



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> C12N 15/12, C12P 21/02 A61K 37/02, C12N 5/12 C07K 15/06	<b>A1</b>	<b>(11) International Publication Number:</b> WO 93/09229 <b>(43) International Publication Date:</b> 13 May 1993 (13.05.93)
<b>(21) International Application Number:</b> PCT/US92/09430 <b>(22) International Filing Date:</b> 2 November 1992 (02.11.92)  <b>(30) Priority data:</b> 787,496                      4 November 1991 (04.11.91)    US 864,692                      7 April 1992 (07.04.92)        US  <b>(71) Applicant:</b> GENETICS INSTITUTE, INC. [US/US]; 87 Cambridge Park Drive, Cambridge, MA 02140 (US).  <b>(72) Inventors:</b> ISRAEL, David ; 117 Anson Road, Concord, MA 01742 (US). WOLFMAN, Neil, M. ; 30 Rolling Lane, Dover, MA 02030 (US).  <b>(74) Agent:</b> KAPINOS, Ellen, J.; Genetics Institute, Inc., 87 Cambridge Park Drive, Cambridge, MA 02140 (US).		<b>(81) Designated States:</b> AU, BR, CA, FI, HU, JP, KR, NO, RU, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> RECOMBINANT BONE MORPHOGENETIC PROTEIN HETERODIMERS, COMPOSITIONS AND METHODS OF USE  <b>(57) Abstract</b>  The present invention relates to methods for producing recombinant heterodimeric BMP proteins useful in the field of treating bone defects, healing bone injury and in wound healing in general. The invention also relates to the recombinant heterodimers and compositions containing them.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SK	Slovak Republic
CI	Côte d'Ivoire	LK	Sri Lanka	SN	Senegal
CM	Cameroon	LU	Luxembourg	SU	Soviet Union
CS	Czechoslovakia	MC	Monaco	TD	Chad
CZ	Czech Republic	MG	Madagascar	TG	Togo
DE	Germany	ML	Mali	UA	Ukraine
DK	Denmark	MN	Mongolia	US	United States of America
ES	Spain			VN	Viet Nam
FI	Finland				

RECOMBINANT BONE MORPHOGENETIC PROTEIN HETERODIMERS,  
COMPOSITIONS AND METHODS OF USE

Field of the Invention

5                   The present invention relates to a series of  
novel recombinant heterodimeric proteins useful in the  
field of treating bone defects, healing bone injury and  
in wound healing in general. The invention also relates  
to methods for obtaining these heterodimers, methods for  
10                   producing them by recombinant genetic engineering  
techniques, and compositions containing them.

Background of the Invention

                  In recent years, protein factors which are  
characterized by bone or cartilage growth inducing  
15                   properties have been isolated and identified. See, e.g.,  
U. S. Patent No. 5,013,649, PCT published application  
WO90/11366; PCT published application WO91/05802 and the  
variety of references cited therein. See, also,  
PCT/US90/05903 which discloses a protein sequence termed  
20                   OP-1, which is substantially similar to human BMP-7, and  
has been reported to have osteogenic activity.

                  A family of individual bone morphogenetic  
proteins (BMPs), termed BMP-2 through BMP-9 have been  
isolated and identified. Incorporated by reference for  
25                   the purposes of providing disclosure of these proteins

and methods of producing them are co-owned, co-pending U. S. Patent Application SN 721,847 and the related applications recited in its preamble. Of particular interest, are the proteins termed BMP-2 and BMP-4, disclosed in the above-referenced application; BMP-7, disclosed in SN 438,919; BMP-5, disclosed in SN 370,547 and SN 356,033; and BMP-6, disclosed in SN 370,544 and SN 347,559; and BMP-8, disclosed in SN 525,357. Additional members of the BMP family include BMP-1, disclosed in SN 655,578; BMP-9, disclosed in SN 720,590; and BMP-3, disclosed in SN 179,197 and PCT publication 89/01464. These applications are incorporated herein by reference for disclosure of these BMPs.

There remains a need in the art for other proteins and compositions useful in the fields of bone and wound healing.

#### Summary of the Invention

In one aspect, the invention provides a method for producing a recombinant heterodimeric protein having bone stimulating activity comprising culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or fragment thereof and a polynucleotide sequence encoding a second selected BMP or fragment thereof. The resulting co-expressed, biologically active heterodimer is isolated from the culture medium.

According to one embodiment of this invention,

the host cell may be co-transfected with one or more vectors containing coding sequences for one or more BMPs. Each BMP polynucleotide sequence may be present on the same vector or on individual vectors transfected into the cell. Alternatively, the BMPs or their fragments may be incorporated into a chromosome of the host cell. Additionally, a single transcription unit may encode single copy of two genes encoding a different BMP.

According to another embodiment of this invention, the selected host cell containing the two polypeptide encoding sequences is a hybrid cell line obtained by fusing two selected, stable host cells, each host cell transfected with, and capable of stably expressing, a polynucleotide sequence encoding a selected first or second BMP or fragment thereof.

In another aspect of the present invention, therefore, there are provided recombinant heterodimeric proteins comprising a protein or fragment of a first BMP in association with a protein or fragment of a second BMP. The heterodimer may be characterized by bone stimulating activity. The heterodimers may comprise a protein or fragment of BMP-2 associated with a protein or fragment of either BMP-5, BMP-6, BMP-7 or BMP-8; or a protein or fragment of BMP-4 associated with a protein or fragment of either BMP-5, BMP-6, BMP-7 or BMP-8. In further embodiments the heterodimers may comprise a protein or fragment of BMP-2 associated with a protein or

fragment of either BMP-1, BMP-3 or BMP-4. BMP-4 may also form a heterodimer in association with BMP-1, BMP-2 or a fragment thereof. Still further embodiments may comprise heterodimers involving combinations of BMP-5, BMP-6, BMP-7 and BMP-8. For example, the heterodimers may comprise BMP-5 associated with BMP-6, BMP-7 or BMP-8; BMP-6 associated with BMP-7 or BMP-8; or BMP-7 associated with BMP-8. These heterodimers may be produced by co-expressing each protein in a selected host cell and isolating the heterodimer from the culture medium.

As a further aspect of this invention a cell line is provided which comprises a first polynucleotide sequence encoding a first BMP or fragment thereof and a second polynucleotide sequence encoding a second BMP or fragment thereof, the sequences being under control of one or more suitable expression regulatory systems capable of co-expressing the BMPs as a heterodimer. The cell line may be transfected with one or more than one polynucleotide molecule. Alternatively, the cell line may be a hybrid cell line created by cell fusion as described above.

Another aspect of the invention is a polynucleotide molecule or plasmid vector comprising a polynucleotide sequence encoding a first selected BMP or fragment thereof and a polynucleotide sequence encoding a second selected BMP or fragment thereof. The sequences are under the control of at least one suitable regulatory

sequence capable of directing co-expression of each protein or fragment. The molecule may contain a single transcription unit containing a copy of both genes, or more than one transcription unit, each containing a copy of a single gene.

As still another aspect of this invention there is provided a method for producing a recombinant dimeric or heterodimeric protein having bone stimulating activity in a prokaryotic cell comprising culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or fragment thereof; culturing a second selected host cell containing a polynucleotide sequence encoding a second selected BMP or fragment thereof; isolating monomeric forms of each BMP protein from the culture medium and co-assembling a monomer of the first protein with a monomer of the second protein. The first protein and the second protein may be the same or different BMPs. The resulting biologically active dimer or heterodimer is thereafter isolated from the mixture. Preferred cells are E. coli.

Thus, as further aspects of this invention recombinant BMP dimers or heterodimers produced in eukaryotic cells are provided, as well as suitable vectors or plasmids, and selected transformed cells useful in such a production method.

Other aspects and advantages of the present invention are described further in the following detailed

description of preferred embodiments of the present invention.

Brief Description of the Figures

Figure 1 provides the DNA and amino acid sequences of human BMP-2 (SEQ ID NOS: 1 and 2).

Figure 2 provides the DNA and amino acid sequences of human BMP-4 (SEQ ID NOS: 3 and 4).

Figure 3 provides the DNA and amino acid sequences of human BMP-7 (SEQ ID NOS: 5 and 6).

Figure 4 provides the DNA and amino acid sequences of human BMP-6 (SEQ ID NOS: 7 and 8).

Figure 5 provides the DNA and amino acid sequences of human BMP-5 (SEQ ID NOS: 9 and 10).

Figure 6 provides the DNA and amino acid sequences of human BMP-8 (SEQ ID NOS: 11 and 12).

Figure 7 provides the DNA sequence of vector pALB2-781 containing the mature portion of the BMP-2 gene (SEQ ID NOS: 13 and 14).

Figure 8 compares the activity of CHO BMP-2 and CHO BMP-2/7 in the W20 alkaline phosphatase assay.

Figure 9 compares the activity of CHO BMP-2 and CHO BMP-2/7 in the BGP (osteocalcin) assay.

Figure 10 provides a comparison of the W-20 activity of E. coli produced BMP-2 and BMP-2/7 heterodimer.

Figure 11 depicts BMP-3 DNA and amino acid sequence.

Figure 12 provides a comparison of BMP-2 and BMP-2/6

in the W-20 assay.

Figure 13 provides a comparison of the in vivo activity of BMP-2/6 and BMP-2.

Figure 14 provides a comparison of BMP-2, BMP-6 and BMP-2/6 in vivo activity.

#### Detailed Description of the Invention

The present invention provides a method for producing recombinant heterodimeric proteins having bone stimulating activity, as well as the recombinant heterodimers themselves, and compositions containing them for bone-stimulating or repairing therapeutic use.

As used throughout this document, the term 'heterodimer' is defined as a biologically-active protein construct comprising the association of two different BMP protein monomers or active fragments thereof joined through at least one covalent, disulfide linkage. A heterodimer of this invention may be characterized by the presence of between one to seven disulfide linkages between the two BMP component strands.

According to the present invention, therefore, a method for producing a recombinant BMP heterodimer according to this invention comprises culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or a biologically active fragment thereof and a polynucleotide sequence encoding a second selected BMP or a fragment thereof. The resulting

co-expressed, biologically active heterodimer is formed within the host cell, secreted therefrom and isolated from the culture medium. Preferred embodiments of methods for producing the heterodimeric proteins of this invention, are described in detail below and in the following examples. Preferred methods of the invention involve known recombinant genetic engineering techniques [See, e.g., Sambrook et al, "Molecular Cloning. A Laboratory Manual:", 2d edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)]. However, other methods, such as conventional chemical synthesis may also be useful in preparing a heterodimer of this invention.

BMP heterodimers generated by this method are produced in a mixture of homodimers and heterodimers. This mixture of heterodimers and homodimers may be separated from contaminants in the culture medium by resort to essentially conventional methods, such as classical protein biochemistry or affinity antibody columns specific for one of the BMPs making up the heterodimer. Additionally, if desired, the heterodimers may be separated from homodimers in the mixture. Such separation techniques allow unambiguous determination of the activity of the heterodimeric species. Example 4 provides one presently employed purification scheme for this purpose.

Preferably the recombinant heterodimers of this

invention produced by these methods involve the BMPs designated human BMP-2, human BMP-4, human BMP-5, human BMP-6, human BMP-7 and BMP-8. However, BMP-3 has also been determined to form an active heterodimer with BMP-2.

5 Other species of these BMPs as well as BMPs than those specifically identified above may also be employed in heterodimers useful for veterinary, diagnostic or research use. However, the human proteins, specifically those proteins identified below, are preferred for human  
10 pharmaceutical uses.

Human BMP-2 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 1. Human BMP-2 proteins are further characterized as  
15 disulfide-linked dimers and homodimers of mature BMP-2 subunits. Recombinantly-expressed BMP-2 subunits include protein species having heterogeneous amino termini. One BMP-2 subunit is characterized by comprising amino acid #249 (Ser) - #396 (Arg) of Figure 1 (SEQ ID NOs: 1 and  
20 2). Another BMP-2 subunit is characterized by comprising amino acid #266 (Thr) - #396 (Arg) of Figure 1. Another BMP-2 subunit is characterized by comprising amino acid #296 (Cys) - #396 (Arg) of Figure 1. A mature BMP-2 subunit is characterized by comprising amino acid #283  
25 (Gln) - #396 (Arg) of Figure 1. This latter subunit is the presently most abundant protein species which results from recombinant expression of BMP-2 (Figure 1).

However, the proportions of certain species of BMP-2 produced may be altered by manipulating the culture conditions. BMP-2 may also include modifications of the sequences of Figure 1, e.g., deletion of amino acids #241-280 and changing amino acid #245 Arg to Ile, among other changes.

As described in detail in United States Patent Application SN 721,847, incorporated by reference herein, human BMP-2 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #356 to #1543 in Figure 1 and recovering and purifying from the culture medium one or more of the above-identified protein species, substantially free from other proteinaceous materials with which it is co-produced. Human BMP-2 proteins are characterized by the ability to induce bone formation. Human BMP-2 also has in vitro activity in the W20 bioassay. Human BMP-2 is further characterized by the ability to induce cartilage formation. Human BMP-2 may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described in the above-referenced application.

Human BMP-4 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 2 (SEQ ID NOS: 3 and 4). Human BMP-4 proteins are

further characterized as disulfide-linked dimers and homodimers of mature BMP-4 subunits. Recombinantly-expressed BMP-4 subunits may include protein species having heterogeneous amino termini. A mature subunit of human BMP-4 is characterized by an amino acid sequence comprising amino acids #293 (Ser) - #408 (Arg) of Figure 2. Other amino termini of BMP-4 may be selected from the sequence of Figure 2. Modified versions of BMP-4, including proteins further truncated at the amino or carboxy termini, may also be constructed by resort to conventional mutagenic techniques.

As disclosed in above-incorporated patent application SN 721,847, BMP-4 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #403 to nucleotide #1626 in Figure 2 and recovering and purifying from the culture medium a protein containing the amino acid sequence from amino acid #293 to #408 as shown in Figure 2, substantially free from other proteinaceous materials with which it is co-produced. BMP-4 proteins are capable of inducing the formation of bone. BMP-4 proteins are capable of inducing formation of cartilage. BMP-4 proteins are further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Human BMP-7 is characterized by containing substantially the entire sequence, or fragments, of the

amino acid sequence and DNA sequence disclosed in Figure 3. Human BMP-7 proteins are further characterized as disulfide-linked dimers and homodimers of mature BMP-7 subunits. Recombinantly-expressed BMP-7 subunits include protein species having heterogeneous amino termini. One BMP-7 subunit is characterized by comprising amino acid #293 (Ser) - #431 (His) of Figure 3 (SEQ ID NOs: 5 and 6). This subunit is the most abundantly formed protein produced by recombinant expression of the BMP-7 sequence. Another BMP-7 subunit is characterized by comprising amino acids #300 (Ser) - #431 (His) of Figure 3. Still another BMP-7 subunit is characterized by comprising amino acids #316 (Ala) - #431 (His) of Figure 3. Other amino termini of BMP-7 may be selected from the sequence of Figure 3. Similarly, modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-7 may also be constructed by resort to conventional mutagenic techniques.

As disclosed in above-incorporated patent application SN 438,919, BMP-7 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #97 to nucleotide #1389 in Figure 3 and recovering and purifying from the culture medium a protein containing the amino acid sequence from amino acid #293 to #431 as shown in Figure 3, substantially free from other proteinaceous or contaminating materials with which it is

co-produced. These proteins are capable of stimulating, promoting, or otherwise inducing cartilage and/or bone formation.

Human BMP-6 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 4. Human BMP-6 proteins are further characterized as disulfide-linked dimers of mature BMP-6 subunits. Recombinantly-expressed BMP-6 subunits may include protein species having heterogeneous amino termini. One BMP-6 subunit is characterized by comprising amino acid #375 (Ser) - #513 (His) of Figure 4 (SEQ ID NOs: 7 and 8). Other amino termini of BMP-6 may be selected from the sequence of Figure 4. Modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-6 may also be constructed by resort to conventional mutagenic techniques.

As described in detail in United States Patent Application SN 490,033, incorporated by reference herein, human BMP-6 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #160 to #1698 in Figure 4 and recovering and purifying from the culture medium a protein comprising amino acid #375 to #513 of Figure 4, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced. Human BMP-6 may be further characterized by

the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Human BMP-5 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 5 (SEQ ID NOs: 9 and 10). Human BMP-5 proteins are further characterized as disulfide-linked dimers of mature BMP-5 subunits. Recombinantly-expressed BMP-5 subunits may include protein species having heterogeneous amino termini. One BMP-5 subunit is characterized by comprising amino acid #329 (Ser) - #454 (His) of Figure 5. Other amino termini of BMP-5 may be selected from the sequence of Figure 5. Modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-5 may also be constructed by resort to conventional mutagenic techniques.

As described in detail in United States Patent Application SN 588,227, incorporated by reference herein, human BMP-5 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #701 to #2060 in Figure 5 and recovering and purifying from the culture medium a protein comprising amino acid #329 to #454 of Figure 5, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced. Human BMP-5 may be further characterized by the ability to demonstrate cartilage and/or bone

formation activity in the rat bone formation assay described in the above-referenced application.

Human BMP-8 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 6. Human BMP-8 proteins may be further characterized as disulfide-linked dimers of mature BMP-8 subunits.

Recombinantly-expressed BMP-8 subunits may include protein species having heterogeneous amino termini. A BMP-8 sequence or subunit sequence comprises amino acid #143 (Ala) - #281 (His) of Figure 6 (SEQ ID NOs: 11 and 12). Other amino termini of BMP-8 may be selected from the sequence of Figure 6. Modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-8 may also be constructed by resort to conventional mutagenic techniques.

As described generally in United States Patent Application SN 525,357, incorporated by reference herein, and as further described herein, human BMP-8 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #1 to #850 in Figure 6 and recovering and purifying from the culture medium a protein comprising amino acid #143 to #281 of Figure 6, or similar amino acid sequences with heterogenous N-termini, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced.

This BMP-8 may also be produced in E. coli by inserting into a vector the sequence encoding amino acid #143 to 281 of Figure 6 with a Met inserted before amino acid #143. Human BMP-8 may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Each above described BMP protein in its native, non-reduced dimeric form may be further characterized by an apparent molecular weight on a 12% Laemmli gel ranging between approximately 28kD to approximately 40kD. Analogs or modified versions of the DNA and amino acid sequences described herein which provide proteins or active fragments displaying bone stimulating or repairing activity in the rat bone formation assay described below in Example 9, are also classified as suitable BMPs for use in this invention, further provided that the proteins or fragments contain one or more Cys residues for participation in disulfide linkages. Useful modifications of these sequences may be made by one of skill in the art with resort to known recombinant genetic engineering techniques. Production of these BMP sequences in mammalian cells produces homodimers, generally mixtures of homodimers having heterologous N termini. Production of these BMP sequences in E.coli produces monomeric protein species.

Thus, according to this invention one recombinant heterodimer of the present invention

comprises the association of a human BMP-2, including, e.g., a monomeric strand from a mature BMP-2 subunit as described above or an active fragment thereof, bound through one or up to seven covalent, disulfide linkages to a human BMP-5 including, e.g., a monomeric strand from a mature BMP-5 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-2, as described above, bound through one or up to seven covalent, disulfide linkages to a human BMP-6, including, e.g., a monomeric strand from a BMP-6 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-2, as described above, bound through one or up to seven covalent, disulfide linkages to a human BMP-7, including, e.g., a monomeric strand of a BMP-7 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-2, as described above, bound through one or up to seven covalent, disulfide linkages to a human BMP-8, including, e.g., a monomeric strand of a BMP-8 subunit as described above or an active fragment thereof.

Still another recombinant heterodimer of the present invention comprises the association of a human BMP-4, including, e.g., a monomeric strand of a BMP-4

subunit as described above or an active fragment thereof,  
bound through one or up to seven covalent, disulfide  
linkages to a human BMP-5, as described above. Another  
recombinant heterodimer of the present invention  
5 comprises the association of a human BMP-4, as described  
above, bound through one or more covalent, disulfide  
linkages to a human BMP-6, as described above. Another  
recombinant heterodimer of the present invention  
comprises the association of a human BMP-4, as described  
10 above bound through one or more covalent, disulfide  
linkages to a human BMP-7, as described above. Another  
recombinant heterodimer of the present invention  
comprises the association of a human BMP-4, as described  
above, bound through one or more covalent, disulfide  
15 linkages to a human BMP-8, as described above.

A further recombinant heterodimer of the  
present invention comprises the association of a human  
BMP-2, including, e.g., a monomeric strand from a mature  
BMP-2 subunit as described above or an active fragment  
20 thereof, bound through at least one disulfide linkage to  
a human BMP-3 including, e.g., a monomeric strand from a  
mature BMP-3 subunit as described above or an active  
fragment thereof. Another recombinant heterodimer of the  
present invention comprises the association of a human  
25 BMP-2, as described above, bound through at least one  
disulfide linkage to a human BMP-4, including, e.g., a  
monomeric strand from a BMP-4 subunit as described above

or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-5, as described above, bound through at least one disulfide linkage to a human BMP-6, including, e.g., a monomeric strand of a BMP-6 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-5, as described above, bound through at least one disulfide linkage to a human BMP-7, including, e.g., a monomeric strand of a BMP-7 subunit as described above or an active fragment thereof. In addition, human BMP-5 may be associated with human BMP-8 bound through at least one disulfide linkage to a human BMP-8 subunit or active fragment thereof.

Still another recombinant heterodimer of the present invention comprises the association of a human BMP-6, including, e.g., a monomeric strand of a BMP-6 subunit as described above or an active fragment thereof, bound through at least one disulfide linkage to a human BMP-7, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-6, as described above, bound through one or more covalent, disulfide linkages to a human BMP-8, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-7, as described above bound through one or more covalent, disulfide linkages to a

human BMP-8, as described above.

The disulfide linkages formed between the monomeric strands of the BMPs may occur between one Cys on each strand. Disulfide linkages may form between two Cys on each BMP. Disulfide linkages may form between three Cys on each BMP. Disulfide linkages may form between four Cys on each BMP. Disulfide linkages may form between five Cys on each BMP. Disulfide linkages may form between six Cys on each BMP. Disulfide linkages may form between seven Cys on each BMP. These disulfide linkages may form between adjacent Cys on each BMP or between only selected Cys interspersed within the respective protein sequence. Various heterodimers having the same BMP component strands may form with different numbers of disulfide linkages. Various heterodimers having the same BMP component strands may form with disulfide bonds at different Cys locations. Different heterodimers encompassed by this invention having the same BMP components may differ based upon their recombinant production in mammalian cells, bacterial cells, insect or yeast cells.

These recombinant heterodimers may be characterized by increased alkaline phosphatase activity in the W20 mouse stromal cell line bioassay (Example 8) compared to the individual BMP homodimers, one strand of which forms each heterodimer. Further, these heterodimers are characterized by greater activity in the

W20 bioassay than is provided by simple mixtures of the individual BMP dimers. Preliminary characterization of heterodimers measured on the W20 bioassay have demonstrated that heterodimers of BMP-2 with BMP-5, BMP-6 or BMP-7 are very active. Similarly, heterodimers of BMP-4 with BMP-5, BMP-6 or BMP-7 are strongly active in the W20 bioassay.

Heterodimers of this invention may also be characterized by activity in bone growth and stimulation assays. For example, a heterodimer of this invention is also active in the rat bone formation assay described below in Example 9. The heterodimers are also active in the osteocalcin bioassay described in Example 8. Other characteristics of a heterodimer of this invention include co-precipitation with anti-BMP antibodies to the two different constituent BMPs, as well as characteristic results on Western blots, high pressure liquid chromatography (HPLC) and on two-dimensional gels, with and without reducing conditions.

One embodiment of the method of the present invention for producing recombinant BMP heterodimers involves culturing a suitable cell line, which has been co-transfected with a DNA sequence coding for expression of a first BMP or fragment thereof and a DNA sequence coding for expression of a second BMP or fragment thereof, under the control of known regulatory sequences. The transformed host cells are cultured and the

heterodimeric protein recovered and purified from the culture medium.

In another embodiment of this method which is the presently preferred method of expression of the heterodimers of this invention, a single host cell, e.g., a CHO DUKX cell, is co-transfected with a first DNA molecule containing a DNA sequence encoding one BMP and a second DNA molecule containing a DNA sequence encoding a second selected BMP. One or both plasmids contain a selectable marker that can be used to establish stable cell lines expressing the BMPs. These separate plasmids containing distinct BMP genes on separate transcription units are mixed and transfected into the CHO cells using conventional protocols. A ratio of plasmids that gives maximal expression of activity in the W20 assay, generally, 1:1, is determined.

For example, as described in detail in Example 3, equal ratios of a plasmid containing the first BMP and a dihydrofolate reductase (DHFR) marker gene and another plasmid containing a second BMP and a DHFR marker gene can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection, electroporation, microinjection, protoplast fusion or lipofection. Individual DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum by conventional means. DHFR+ cells containing increased gene copies can be selected

for propagation in increasing concentrations of methotrexate (MTX) (e.g. sequential steps in 0.02, 0.1, 0.5 and 2.0 uM MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982); and Kaufman et al, Mol. Cell Biol., 5:1750 (1983).

Expression of the heterodimer or at least one BMP linked to DHFR should increase with increasing levels of MTX resistance. Cells that stably express either or both BMP/DHFR genes will survive. However at a high frequency, cell lines stably incorporate and express both plasmids that were present during the initial transfection. The conditioned medium is thereafter harvested and the heterodimer isolated by conventional methods and assayed for activity. This approach can be employed with DHFR-deficient cells.

As an alternative embodiment of this method, a DNA molecule containing one selected BMP gene may be transfected into a stable cell line which already expresses another selected BMP gene. For example as described in detail in Example 3 below, a stable CHO cell line expressing BMP-7 with the DHFR marker (designated 7MB9) [Genetics Institute, Inc] is transfected with a plasmid containing BMP-2 and a second selectable marker gene, e.g., neomycin resistance (Neo). After transfection, the cell is cultured and suitable cells selected by treatment with MTX and the antibiotic, G-418. Surviving cells are then screened for the expression of

the heterodimer. This expression system has the advantage of permitting a single step selection.

Alternative dual selection strategies using different cell lines or different markers can also be used. For example, the use of an adenosine deaminase (ADA) marker to amplify the second BMP gene in a stable CHO cell line expressing a different BMP with the DHFR marker may be preferable, since the level of expression can be increased using deoxycoformycin (DCF)-mediated gene amplification. (See the ADA containing plasmid described in Example 1). Alternatively, any BMP cell line made by first using this marker can then be the recipient of a second BMP expression vector containing a distinct marker and selected for dual resistance and BMP coexpression.

Still another embodiment of a method of expressing the heterodimers of this invention includes transfecting the host cell with a single DNA molecule encoding multiple genes for expression either on a single transcription unit or on separate transcription units. Multicistronic expression involves multiple polypeptides encoded within a single transcript, which can be efficiently translated from vectors utilizing a leader sequence, e.g., from the EMC virus, from poliovirus, or from other conventional sources of leader sequences. Two BMP genes and a selectable marker can be expressed within a single transcription unit. For example, vectors

containing the configuration BMPx-EMC-BMPy-DHFR or BMPx-EMC-BMPy-EMC-DHFR can be transfected into CHO cells and selected and amplified using the DHFR marker. A plasmid may be constructed which contains DNA sequences encoding  
5 two different BMPs, one or more marker genes and a suitable leader or regulatory sequence on a single transcription unit.

Similarly, host cells may be transfected with a single plasmid which contains separate transcription  
10 units for each BMP. A selectable marker, e.g., DHFR, can be contained on a another transcription unit, or alternatively as the second cistron on one or both of the BMP genes. These plasmids may be transfected into a selected host cell for expression of the heterodimer, and  
15 the heterodimer isolated from the cells or culture medium as described above.

Another embodiment of this expression method involves cell fusion. Two stable cell lines which express selected BMPs, such as a cell line expressing  
20 BMP-2 (e.g., 2EG5) and a cell line expressing BMP-7 (e.g., 7MB9), developed using the DHFR/MTX gene amplification system and expressing BMP at high levels, as described in Example 1 and in the above incorporated U.S. applications, can be transfected with one of several  
25 dominant marker genes (e.g., neo', hygromycin', GPT). After sufficient time in coculture (approximately one day) one resultant cell line expressing one BMP and a

dominant marker can be fused with a cell line expressing a different BMP and preferably a different marker using a fusigenic reagent, such as polyethylene glycol, Sendai virus or other known agent.

5           The resulting cell hybrids expressing both dominant markers and DHFR can be selected using the appropriate culture conditions, and screened for coexpression of the BMPs or their fragments. The selected hybrid cell contains sequences encoding both  
10 selected BMPs, and the heterodimer is formed in the cell and then secreted. The heterodimer is obtained from the conditioned medium and isolated and purified therefrom by conventional methods (see e.g., Example 4). The resulting heterodimer may be characterized by methods  
15 described herein.

Cell lines generated from the approaches described above can be used to produce co-expressed, heterodimeric BMP polypeptides. The heterodimeric proteins are isolated from the cell medium in a form  
20 substantially free from other proteins with which they are co-produced as well as from other contaminants found in the host cells by conventional purification techniques. The presently preferred method of production is co-transfection of different vectors into CHO cells  
25 and methotrexate-mediated gene amplification. Stable cell lines may be used to generate conditioned media containing recombinant BMP that can be purified and

assayed for in vitro and in vivo activities. For example, the resulting heterodimer-producing cell lines obtained by any of the methods described herein may be screened for activity by the assays described in Examples 8 and 9, RNA expression, and protein expression by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The above-described methods of co-expression of the heterodimers of this invention utilize suitable host cells or cell lines. Suitable cell preferably include mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U. S. Patent 4,419,446. Other suitable mammalian cell lines are the CV-1 cell line, BHK cell lines and the 293 cell line. The monkey COS-1 cell line is presently believed to be inefficient in BMP heterodimer production.

Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention, e.g., Saccharomyces cerevisiae. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g.,

Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another method for producing a biologically active heterodimeric protein of this invention may be employed where the host cells are microbial, preferably bacterial cells, in particular E. coli. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

This method, which may be employed to produce monomers and dimers (both homodimers and heterodimers) is described in European Patent Application No. 433,225, incorporated herein by reference. Briefly, this process involves culturing a microbial host comprising a nucleotide sequence encoding the desired BMP protein linked in the proper reading frame to an expression control sequence which permits expression of the protein and recovering the monomeric, soluble protein. Where the protein is insoluble in the host cells, the water-insoluble protein fraction is isolated from the host cells and the protein is solubilized. After chromatographic purification, the solubilized protein is subjected to selected conditions to obtain the biologically active dimeric configuration of the protein. This process, which may be employed to produce the heterodimers of this invention, is described specifically

in Example 7, for the production of a BMP-2 homodimer.

Another aspect of the present invention provides DNA molecules or plasmid vectors for use in expression of these recombinant heterodimers. These plasmid vectors may be constructed by resort to known methods and available components known to those of skill in the art. In general, to generate a vector useful in the methods of this invention, the DNA encoding the desired BMP protein is transferred into one or more appropriate expression vectors suitable for the selected host cell.

It is presently contemplated that any expression vector suitable for efficient expression in mammalian cells may be employed to produce the recombinant heterodimers of this invention in mammalian host cells. Preferably the vectors contain the selected BMP DNA sequences described above and in the Figures, which encode selected BMP components of the heterodimer. Alternatively, vectors incorporating modified sequences as described in the above-referenced patent applications are also embodiments of the present invention and useful in the production of the vectors.

In addition to the specific vectors described in Example 1, one skilled in the art can construct mammalian expression vectors by employing the sequence of Figures 1-6 or other DNA sequences containing the coding sequences of Figures 1-6 (SEQ ID NOs: 1, 3, 5, 7, 9 and

11), or other modified sequences and known vectors, such as pCD [Okayama et al, Mol. Cell Biol., 2:161-170 (1982)] and pJL3, pJL4 [Gough et al, EMBO J., 4:645-653 (1985)]. The BMP DNA sequences can be modified by removing the non-coding nucleotides on the 5' and 3' ends of the coding region. The deleted non-coding nucleotides may or may not be replaced by other sequences known to be beneficial for expression. The transformation of these vectors into appropriate host cells as described above can produce desired heterodimers.

One skilled in the art could manipulate the sequences of Figures 1-6 by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with e.g., yeast or insect regulatory sequences, to create vectors for intracellular or extracellular expression by yeast or insect cells. [See, e.g., procedures described in published European Patent Application 155,476] for expression in insect cells; and procedures described in published PCT application WO86/00639 and European Patent Application EPA 123,289 for expression in yeast cells].

Similarly, bacterial sequences and preference codons may replace sequences in the described and exemplified mammalian vectors to create suitable expression systems for use in the production of BMP monomers in the method described above. For example, the coding sequences could be further manipulated (e.g.,

ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering nucleotides therein by other known techniques). The modified BMP coding sequences could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al, Proc. Natl. Acad. Sci. USA, 77:5230-5233 (1980). The exemplary bacterial vector could then be transformed into bacterial host cells and BMP heterodimers expressed thereby. An exemplary vector for microbial, e.g., bacterial, expression is described below in Example 7.

Other vectors useful in the methods of this invention may contain multiple genes in a single transcription unit. For example, a proposed plasmid p7E2D contains the BMP-7 gene followed by the EMC leader sequence, followed by the BMP-2 gene, followed by the DHFR marker gene. Another example is plasmid p7E2ED which contains the BMP-7 gene, the EMC leader, the BMP-2 gene, another EMC leader sequence and the DHFR marker gene. Alternatively, the vector may contain more than one transcription unit. As one example, the plasmid p2ED7ED contains a transcription unit for BMP-2 and a separate transcription unit for BMP-7, i.e., BMP-2-EMC-DHFR and BMP-7-EMC-DHFR. Alternatively, each transcription unit on the plasmid may contain a different marker gene. For example, plasmid p2EN7ED contains BMP-2-EMC-Neo and BMP-7-EMC-DHFR.

Additionally the vectors also contain appropriate expression control sequences which are capable of directing the replication and expression of the BMP in the selected host cells. Useful regulatory sequences for such vectors are known to one of skill in the art and may be selected depending upon the selected host cells. Such selection is routine and does not form part of the present invention. Similarly, the vectors may contain one or more selection markers, such as the antibiotic resistance gene, Neo or selectable markers such as DHFR and ADA. The presently preferred marker gene is DHFR. These marker genes may also be selected by one of skill in the art.

Once they are expressed by one of the methods described above, the heterodimers of this invention may be identified and characterized by application of a variety of assays and procedures. A co-precipitation (immunoprecipitation) assay may be performed with antibodies to each of the BMPs forming the heterodimer. Generally antibodies for this use may be developed by conventional means, e.g., using the selected BMP, fragments thereof, or synthetic BMP peptides as antigen. Antibodies employed in assays are generally polyclonal antibodies made from individual BMP peptides or proteins injected into rabbits according to classical techniques. This assay is performed conventionally, and permits the identification of the heterodimer, which is precipitated

by antibodies to both BMP components of the heterodimer. In contrast, only one of the two antibodies causes precipitation of any homodimeric form which may be produced in the process of producing the heterodimer.

5 Another characterizing assay is a Western assay, employing a precipitating antibody, a probing antibody and a detecting antibody. This assay may also be performed conventionally, by using an antibody to one of the BMPs to precipitate the dimers, which are run on  
10 reducing SDS-PAGE for Western analysis. An antibody to the second BMP is used to probe the precipitates on the Western gel for the heterodimer. A detecting antibody, such as a goat-antirabbit antibody labelled with horseradish peroxidase (HRP), is then applied, which will  
15 reveal the presence of one of the component subunits of the heterodimer.

Finally, the specific activity of the heterodimer may be quantitated as described in detail in Example 6. Briefly, the amount of each BMP is  
20 quantitated using Western blot analysis or pulse labelling and SDS-PAGE analysis in samples of each BMP homodimer and the heterodimer. The W20 activity is also determined as described specifically in Example 8. The relative specific activities may be calculated by the  
25 formula: W20 alkaline phosphatase activity/amount of BMP on Western blot or by fluorography. As one example, this formula has been determined for the BMP-2/7 heterodimer,

demonstrating that the heterodimer has an estimated 5 to 50 fold higher specific activity than the BMP-2 homodimer.

The heterodimers of the present invention may have a variety of therapeutic and pharmaceutical uses, e.g., in compositions for wound healing, tissue repair, and in similar compositions which have been indicated for use of the individual BMPs. Increased potency of the heterodimers over the individual BMPs may permit lower dosages of the compositions in which they are contained to be administered to a patient in comparison to dosages of compositions containing only a single BMP. A heterodimeric protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a heterodimeric protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A heterodimeric protein of this invention may be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an

environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Heterodimeric polypeptides of the invention may also be useful in the treatment of osteoporosis. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g., European Patent Applications 148,155 and 169,016 for discussions thereof.

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See, e.g., PCT Publication WO84/01106 incorporated by reference herein for discussion of wound healing and related tissue repair).

Additionally, the proteins of the invention may increase neuronal survival and therefore be useful in transplantation and treatment of conditions exhibiting a decrease in neuronal survival.

In view of the usefulness of the heterodimers, therefore, a further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal diseases. In addition, the invention comprises therapeutic methods and compositions for wound healing and tissue repair. Such compositions comprise a therapeutically effective amount of a heterodimeric protein of the invention in admixture

with a pharmaceutically acceptable vehicle, carrier or matrix. The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is  
5 within the skill of the art.

It is expected that the proteins of the invention may act in concert with other related proteins and growth factors. Therapeutic methods and compositions of the invention therefore comprise a therapeutic amount  
10 of a heterodimeric protein of the invention with a therapeutic amount of at least one of the other BMP proteins disclosed in co-owned and concurrently filed U. S. applications described above. Such combinations may comprise separate molecules of the BMP proteins or other  
15 heteromolecules of the present invention.

In further compositions, heterodimeric proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents  
20 include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently  
25 valuable for veterinary applications due to the lack of species specificity in BMP proteins. Particularly domestic animals and thoroughbred horses, in addition to

humans, are desired patients for such treatment with heterodimeric proteins of the present invention.

The therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than the heterodimeric proteins of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the heterodimeric BMP composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the heterodimeric protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical

properties, cosmetic appearance and interface properties. The particular application of the heterodimeric BMP compositions will define the appropriate formulation. Potential matrices for the compositions may be

5 biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further

10 matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the

15 above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle

20 shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to

25 utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the BMP compositions from dissassociating from the matrix.

The dosage regimen of a heterodimeric protein-containing pharmaceutical composition will be determined by the attending physician considering various factors which modify the action of the heterodimeric proteins, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the BMP proteins in the heterodimer and any additional BMP or other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

The following examples are illustrative of the present invention and do not limit its scope.

#### EXAMPLE 1 - BMP Vector Constructs and Cell Lines

##### A. BMP-2 Vectors

The mammalian expression vector pMT2 CXM is a derivative of p91023 (b) [Wong et al, Science, 228:810-815 (1985)] differing from the latter in that it

contains the ampicillin resistance gene (Amp) in place of the tetracycline resistance gene (Tet) and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 CXM have been described [R. J. Kaufman, Proc. Natl. Acad. Sci. USA, 82:689-693 (1985)] and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122, excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form. Plasmid pMT2 can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods.

Plasmid pMT2 CXM is then constructed using loopout/in mutagenesis [Morinaga et al, Biotechnology, 84:636 (1984)]. This removes bases 1075 to 1145 relative to the HindIII site near the SV40 origin of replication and enhancer sequences of pMT2. In addition it inserts the following sequence:

38

5

10

15

3

(SEQ ID NO: 16)

25

The SalI fragment of this clone was subcloned into the expression vector pMT23, yielding the vector pMT23-BMP2 $\Delta$ UT. Restriction enzyme sites flank the BMP-2 coding region in the sequence PstI-EcoRI-SalI-BMP-2 cDNA-SalI-EcoRI-XhoI.

The expression plasmid pED4 [Kaufman et al, Nucl. Acids Res., 19:4485-4490 (1991)] was linearized by digestion with EcoRI and treated with calf intestinal phosphatase. The BMP-2 cDNA gene was excised from pMT23-BMP2 $\Delta$ UT by digestion with EcoRI and recovery of the 1.2 kb fragment by electrophoresis through a 1.0% low melt agarose gel. The linearized pED4 vector and the EcoRI BMP-2 fragment were ligated together, yielding the BMP-2 expression plasmid pBMP2 $\Delta$ -EMC.

Another vector pBMP-2 $\Delta$ -EN contains the same sequences contained within the vector pBMP2 $\Delta$ -EMC, except the DHFR gene has been replaced by conventional means with the neomycin resistance gene from the Tn5 transposable element.

#### B. BMP4 Vectors

A BMP-4 cDNA sequence set forth in Figure 2 (SEQ ID NO: 3), in which the 3' untranslated region is removed, is made via heteroduplex mutagenesis with the mutagenic oligonucleotide:

5' GGATGTGGGTGCCGCTGACTCTAGAGTCGACGGAATTC 3'  
End EcoRI  
(SEQ ID NO: 17)

5 This deletes all of the sequences 3' to the translation  
terminator codon of the BMP-4 cDNA, juxtaposing this  
terminator codon and the vector polylinker sequences.  
This step is performed in an SP65 vector [Promega  
Biotech] and may also be conveniently performed in pMT2-  
derivatives containing the BMP-4 cDNA similar to the BMP2  
10 vectors described above. The 5' untranslated region is  
removed using the restriction endonuclease BsmI, which  
cleaves within the eighth codon of BMP-4 cDNA.

Reconstruction of the first eight codons  
is accomplished by ligation to oligonucleotides:

**15**            EcoRI Initiator                          BsmI  
5'     AATTCACCATGATTCTGGTAACCGAATGCT     3'     (SEQ ID NO: 18)

and

3' GTGGTACTAAGGACCATTTGGCTTAC 5' (SEQ ID NO: 19)

These oligonucleotides form a duplex which has a BsmI  
complementary cohesive end capable of ligation to the  
BsmI restricted BMP-4 cDNA, and it has an EcoRI  
complementary cohesive end capable of ligation to the  
EcoRI restricted vector pMT2. Thus the cDNA for BMP-4  
with the 5' and 3' untranslated regions deleted, and  
retaining the entire encoding sequence is contained  
within an EcoRI restriction fragment of approximately 1.2  
kb.

The pMT2 CXM plasmid containing this BMP-4

sequence is designated pXMBMP-4ΔUT. It is digested with EcoRI in order to release the BMP-4 cDNA containing insert from the vector. This insert is subcloned into the EcoRI site of the mammalian expression vector pED4, resulting pBMP4Δ-EMC.

C. BMP-5 Vectors

A BMP-5 cDNA sequence comprising the nucleotide sequence from nucleotide #699 to #2070 of Fig. 5 (SEQ ID NO: 9) is specifically amplified as follows.

The oligonucleotides CGACCTGCAGCCACCATGCATCTGACTGTA (SEQ ID NO: 20) and TGCCTGCAGTTTAATATTAGTGGCAGC (SEQ ID NO: 21) are utilized as primers to allow the amplification of nucleotide sequence #699 to #2070 of Fig. 5 from the BMP-5 insert of λ-ZAP clone U2-16 [ATCC #68109]. This

procedure introduces the nucleotide sequence CGACCTGCAGCCACC (SEQ ID NO: 22) immediately preceding nucleotide #699 and the nucleotide sequence CTGCAGGCA immediately following nucleotide #2070. The addition of these sequences results in the creation of PstI

restriction endonuclease recognition sites at both ends of the amplified DNA fragment. The resulting amplified DNA product of this procedure is digested with the restriction endonuclease PstI and subcloned into the PstI site of the pMT2 derivative pMT21 [Kaufman, Nucl. Acids Res., 19:4485-4490 (1991)]. The resulting clone is designated H5/5/pMT.

The insert of H5/5/pMT is excised by PstI

digestion and subcloned into the plasmid vector pSP65 [Promega Biotech] at the PstI site, resulting in plasmid BMP5/SP6. BMP5/SP6 and U2-16 are digested with the restriction endonucleases NsiI and NdeI to excise the portion of their inserts corresponding to nucleotides #704 to #1876 of Fig. 5. The resulting 1173 nucleotide NsiI-NdeI fragment of clone U2-16 is ligated into the NsiI-NdeI site of BMP5/SP6 from which the corresponding 1173 nucleotide NsiI-NdeI fragment had been removed. The resulting clone is designated BMP5mix/SP65.

Direct DNA sequence analysis of BMP5mix/SP65 is performed to confirm identity of the nucleotide sequences produced by the amplification to those set forth in Fig. 5. The clone BMP5mix/SP65 is digested with the restriction endonuclease PstI resulting in the excision of an insert comprising the nucleotides #699 to #2070 of Fig. 5 and the additional sequences containing the PstI recognition sites as described above. The resulting 1382 nucleotide PstI fragment is subcloned into the PstI site of the pMT2 derivative pMT21. This clone is designated BMP5mix/pMT21#2.

The same fragment is also subcloned into the PstI site of pED4 to yield the vector designated BMP5mix-EMC-11.

#### 25 D. BMP-6 Vectors

A BMP-6 cDNA sequence comprising the nucleotide sequence from nucleotide #160 to #1706 of

Fig. 4 (SEQ ID NO: 7) is produced by a series of techniques known to those skilled in the art. The clone BMP6C35 [ATCC 68245] is digested with the restriction endonucleases ApaI and TaqI, resulting in the excision of a 1476 nucleotide portion of the insert comprising nucleotide #231 to #1703 of Fig. 4. Synthetic oligonucleotides with SalI restriction endonuclease site converters are designed to replace those nucleotides corresponding to #160 to #230 and #1704 to #1706 which are not contained in the 1476 ApaI-TaqI fragment of the BMP-6 cDNA sequence.

Oligonucleotide/SalI converters conceived to replace the missing 5'

(TCGACCCACCATGCCGGGGCTGGGGCGGAGGGCGCAGTGGCTGT  
GCTGGTGGTGGGGGCTGTGCTGCAGCTGCTGCGGGCC (SEQ ID NO: 23) and  
CGCAGCAGCTGCACAGCAGCCCCACCACCAGCACAGCCACTGCGCCCTCCGCCCCA  
GCCCCGGCATGGTGGG) (SEQ ID NO: 24) and 3' (TCGACTGGTTT  
(SEQ ID NO: 25) and CGAAACCAG (SEQ ID NO: 26) ) sequences  
are annealed to each other independently. The annealed  
5' and 3' converters are then ligated to the 1476  
nucleotide ApaI-TaqI described above, creating a 1563  
nucleotide fragment comprising the nucleotide sequence  
from #160 to #1706 of Fig. 4 and the additional sequences  
contrived to create SalI restriction endonuclease sites  
at both ends. The resulting 1563 nucleotide fragment is  
subcloned into the SalI site of pSP64 [Promega Biotech,  
Madison, WI]. This clone is designated BMP6/SP64#15.

DNA sequence analysis of BMP6/SP64#15 is performed to confirm identity of the 5' and 3' sequences replaced by the converters to the sequence set forth in Fig. 4. The insert of BMP6/SP64#15 is excised by digestion with the restriction endonuclease SalI. The resulting 1563 nucleotide SalI fragment is subcloned into the XhoI restriction endonuclease site of pMT21 and designated herein as BMP6/pMT21.

The PstI site of pED4 is converted to a SalI site by digestion of the plasmid with PstI and ligation to the converter oligonucleotides:

5'-TCGACAGGCTCGCCTGCA-3' (SEQ ID NO: 27) and  
3'-GTCCGAGCGG-5' (SEQ ID NO: 28).

The above 1563 nucleotide SalI fragment is also subcloned into the SalI site of this pED4 vector, yielding the expression vector BMP6/EMC.

#### E. BMP-7 Vectors

A BMP-7 sequence comprising the nucleotide sequence from nucleotide #97 to #1402 of Fig. 3 (SEQ ID NO: 5) is specifically amplified as follows. The oligonucleotides CAGGTCGACCCACCATGCACGTGCGCTCA (SEQ ID NO: 29) and TCTGTGACCTCGGAGGAGCTAGTGGC (SEQ ID NO: 30) are utilized as primers to allow the amplification of nucleotide sequence #97 to #1402 of Fig. 3 from the insert of clone PEH7-9 [ATCC #68182]. This procedure generates the insertion of the nucleotide sequence CAGGTCGACCCACC immediately preceding nucleotide #97 and

the insertion of the nucleotide sequence GTCGACAGA immediately following nucleotide #1402. The addition of these sequences results in the creation of a SalI restriction endonuclease recognition site at each end of the amplified DNA fragment. The resulting amplified DNA product of this procedure is digested with the restriction endonuclease SalI and subcloned into the SalI site of the plasmid vector pSP64 [Promega Biotech, Madison, WI] resulting in BMP7/SP6#2.

The clones BMP7/SP6#2 and PEH7-9 are digested with the restriction endonucleases NcoI and StuI to excise the portion of their inserts corresponding to nucleotides #363 to #1081 of Fig. 3. The resulting 719 nucleotide NcoI-StuI fragment of clone PEH7-9 is ligated into the NcoI-StuI site of BMP7/SP6#2 from which the corresponding 719 nucleotide fragment is removed. The resulting clone is designated BMP7mix/SP6.

Direct DNA sequence analysis of BMP7mix/SP6 confirmed identity of the 3' region to the nucleotide sequence from #1082 to #1402 of Fig. 3, however the 5' region contained one nucleotide misincorporation.

Amplification of the nucleotide sequence (#97 to #1402 of Fig. 3) utilizing PEH7-9 as a template is repeated as described above. The resulting amplified DNA product of this procedure is digested with the restriction endonucleases SalI and PstI. This digestion results in the excision of a 747 nucleotide fragment

comprising nucleotide #97 to #833 of Fig. 3 plus the additional sequences of the 5' priming oligonucleotide used to create the SalI restriction endonuclease recognition site described earlier. This 747 SalI-PstI fragment is subcloned into a SalI-PstI digested pSP65 [Promega Biotech, Madison, WI] vector resulting in 5'BMP7/SP65. DNA sequence analysis demonstrates that the insert of the 5'BMP7/SP65#1 comprises a sequence identical to nucleotide #97 to #362 of Fig. 3.

10           The clones BMP7mix/SP6 and 5'BMP7/SP65 are digested with the restriction endonucleases SalI and NcoI. The resulting 3' NcoI-SalI fragment of BMP7mix/SP6 comprising nucleotides #363 to #1402 of Fig. 3 and 5' SalI-NcoI fragment of 5'BMP7/SP65 comprising nucleotides  
15           #97 to #362 of Fig. 3 are ligated together at the NcoI restriction sites to produce a 1317 nucleotide fragment comprising nucleotides #97 to #1402 of Fig. 3 plus the additional sequences derived from the 5' and 3' oligonucleotide primers which allows the creation of SalI  
20           restriction sites at both ends of this fragment.

          This 1317 nucleotide SalI fragment is ligated into the SalI site of the pMT2 derivative pMT2Cla-2. pMT2Cla-2 is constructed by digesting pMT21 with EcoRV and XhoI, treating the digested DNA with Klenow  
25           fragment of DNA polymerase I and ligating ClaI linkers (NEBio Labs, CATCGATG). This removes bases 2171 to 2420 starting from the HindIII site near the SV40 origin of

replication and enhancer sequences of pMT2 and introduces a unique ClaI site, but leaves the adenovirus VAI gene intact, resulting in pMT2Cla-2. This clone is designated BMP-7-pMT2.

5                   The insert of BMP-7-pMT2 is excised by digestion with the restriction endonuclease SalI. The resulting 1317 nucleotide SalI fragment is subcloned into the XhoI restriction endonuclease site of pMT21 to yield the clone BMP-7/pMT21. This SalI fragment is also  
10                   subcloned into the SalI site of the pED4 vector in which the PstI site was converted into a SalI site as described above, resulting in the vector pBMP7/EMC#4.

#### F. BMP-8 Vectors

                  At present no mammalian BMP-8 vectors have  
15                   been constructed. However, using the sequence of Figure 6 (SEQ ID NO: 11), it is contemplated that vectors similar to those described above for the other BMPs may be readily constructed. A bacterial expression vector similar to the BMP-2 vector described in detail in  
20                   Example 7 may also be constructed for BMP-8, by introducing a Met before the amino acid #284 Ala of Fig. 6. This sequence of BMP-8 is inserted into the vector pALBP2-781 in place of the BMP-2 sequence. See Example  
25                   7.

#### G. BMP Vectors Containing the Adenosine Deaminase (Ada) Marker

BMP genes were inserted into the vector

pMT3SV2Ada [R. J. Kaufman, Meth. Enz., 185:537-566 (1990)] to yield expression plasmids containing separate transcription units for the BMP cDNA gene and the selectable marker Ada. pMT3SV2Ada contains a polylinker with recognition sites for the enzymes PstI, EcoRI, Sall and XbaI that can be used for insertion of and expression of genes (i.e. BMP) in mammalian cells. In addition, the vector contains a second transcription unit encoding Ada which serves as a dominant and amplifiable marker in mammalian cells.

To construct expression vectors for BMP-5, BMP-6 and BMP-7, individually, the same general method was employed. The gene for BMP 5 (Fig. 5), 6 (Fig. 4) or 7 (Fig. 3) was inserted into the polylinker essentially as described above for the pED4 vector. These vectors can be used for transfection into CHO DUKX cells and subsequent selection and amplification using the Ada marker as previously described [Kaufman et al, Proc. Natl. Acad. Sci. USA, 83:3136-3140 (1986)]. Since each such vector does not contain a DHFR gene, the resultant transformed cells remain DHFR negative and can be subsequently transfected with a second vector containing a different BMP in conjunction with DHFR and amplified with methotrexate.

Alternatively, the pMT3SV2Ada/BMP vectors can be used to transfect stable CHO cell lines previously transfected with a different BMP gene and amplified using

the DHFR/methotrexate system. The resultant transfectants can be subsequently amplified using the Ada system, yielding cell lines that coexpress two different BMP genes, and are amplified using both the DHFR and Ada markers.

#### H. BMP-Expressing Mammalian Cell Lines

At present, the most desirable mammalian cell lines for use in producing the recombinant homodimers and heterodimers of this invention are the following. These cell lines were prepared by conventional transformation of CHO cells using vectors described above.

The BMP-2 expressing cell line 2EG5 is a CHO cell stably transformed with the vector pBMP2delta-EMC.

The BMP-4 expressing cell line 4E9 is a CHO cell stably transformed with the vector pBMP4delta-EMC.

The BMP-5 expressing cell line 5E10 is a CHO cell stably transformed with the vector BMP5mix-EMC-11 (at a amplification level of 2 micromolar MTX).

The BMP-6 expressing cell line 6HG8 is a CHO cell stably transformed with the vector BMP6/EMC.

The BMP-7 expressing cell line 7MB9 is a CHO cell stably transformed with the vector BMP7/pMT21.

#### EXAMPLE 2 - TRANSIENT EXPRESSION OF BMP HETERODIMERS

The heterodimers of the present invention may be prepared by co-expression in a transient expression system for screening in the assays of Example 8 by two different techniques as follows.

5 In the first procedure, the pMT2-derived and EMC-derived expression plasmids described in Example 1 and other similarly derived vectors were constructed which encoded, individually, BMP-2 through BMP-7, and transforming growth factor-beta ( $TGF\beta 1$ ). All  
10 combinations of pairs of plasmids were mixed in equal proportion and used to co-transfect CHO cells using the DEAE-dextran procedure [Sompayrac and Danna, Proc. Natl. Acad. Sci. USA, 78:7575-7578 (1981); Luthman and Magnusson, Nucl. Acids Res., 11:1295-1308 (1983)]. The  
15 cells are grown in alpha Minimal Essential Medium ( $\alpha$ -MEM) supplemented with 10% fetal bovine serum, adenosine, deoxyadenosine, thymidine (100  $\mu$ g/ml each), pen/strep, and glutamine (1 mM).

The addition of compounds such as heparin,  
20 suramin and dextran sulfate are desirable in growth medium to increase the amounts of BMP-2 present in the conditioned medium of CHO cells. Similarly responsive to such compounds is BMP-5. Therefore, it is expected that these compounds will be added to growth medium for any  
25 heterodimer containing these BMP components. Other BMPs may also be responsive to the effects of these compounds, which are believed to inhibit the interaction of the

mature BMP molecules with the cell surface.

The following day, fresh growth medium, with or without 100  $\mu$ g/ml heparin, was added. Twenty-four hours later, conditioned medium was harvested.

5           In some experiments, the conditioned medium was collected minus heparin for the 24-48 hour period post-transfection, and the same plates were then used to generate conditioned medium in the presence of heparin 48-72 hour post-transfection. Controls included  
10   transfecting cells with expression plasmids lacking any BMP sequences, transfecting cells with plasmids containing sequences for only a single BMP, or mixing conditioned medium from cells transfected with a single BMP with conditioned medium from cells transfected with a  
15   different BMP.

Characterizations of the coexpressed heterodimer BMPs in crude conditioned media, which is otherwise not purified, provided the following results. Transiently coexpressed BMP was assayed for induction of  
20   alkaline phosphatase activity on W20 stromal cells, as described in Example 8.

Co-expression of BMP-2 with BMP-5, BMP-6 and BMP-7, and BMP-4 with BMP-5, BMP-6 and BMP-7 yielded more alkaline phosphatase inducing activity in the W20 assay  
25   than either of the individual BMP homodimers alone or mixtures of homodimers, as shown below. Maximal activity (in vitro), was obtained when BMP-2 was coexpressed with

BMP-7. Increased activity was also found the heterodimers BMP-2/5; BMP-2/6; BMP-4/5; BMP-4/6; and BMP-4/7.

	Condition Medium						
	TGF- $\beta$	BMP-7	BMP-6	BMP-5	BMP-4	BMP-3	BMP-2
BMP-2	33	240	99	89	53	9	29
BMP-3	-	-	-	-	14	-	
BMP-4	12	115	25	22	24		
BMP-5	-	-	-	-			
BMP-6	-	-	-				
BMP-7	-	-					
TGF- $\beta$	-						

	Condition Medium + heparin						
	TGF- $\beta$	BMP-7	BMP-6	BMP-5	BMP-4	BMP-3	BMP-2
BMP-2	88	454	132	127	70	77	169
BMP-3	-	-	-	-	7	-	
BMP-4	7	119	30	41	37		
BMP-5	-	-	-	-			
BMP-6	-	-	-				
BMP-7	-	-					
TGF- $\beta$	-						

Units: 1 unit of activity is equivalent to that of 1 ng/ml of rhBMP-2.  
 -: indicates activity below the detection limit of the assay.

These BMP combinations were subsequently expressed using various ratios of expression plasmids (9:1, 3:1, 1:1, 1:3, 1:9) during the CHO cell transient transfection. The performance of this method using plasmids containing BMP-2 and plasmids containing BMP-7 at plasmid number ratios ranging from 9:1 to 1:9, respectively, demonstrated that the highest activity in

the W20 assay was obtained when approximately the same number of plasmids of each BMP were transfected into the host cell. Ratios of BMP-2 to BMP-7 plasmids of 3:1 to 1:3, respectively, also resulted in increased activity in W20 assay in comparison to host cells transfected with plasmids containing only a single BMP. However, these latter ratios produced less activity than the 1:1 ratio.

Similar ratios may be determined by one of skill in the art for heterodimers consisting of other than BMP-2 and BMP-7. For example, preliminary work on the heterodimer formed between BMP-2 and BMP-6 has indicated that a preferred ratio of plasmids for co-transfection is 3:1, respectively. The determination of preferred ratios for this method is within the skill of the art.

As an alternative means to transiently generate coexpressed BMPs, the stable CHO cell lines identified in Example 1 expressing each BMP-2, BMP-4, BMP-5, BMP-6 and BMP-7, are cocultured for one day, and are then fused with 46.7% polyethylene glycol (PEG). One day post-fusion, fresh medium is added and the heterodimers are harvested 24 hours later for the W20 assay, described in Example 8. The assay results were substantially similar to those described immediately above.

Therefore, all combinations of BMP-2 or 4 coexpressed with either BMP-5, 6 or 7 yielded greater activity than any of the BMP homodimers alone. In

control experiments where each BMP homodimer was expressed alone and conditioned media mixed post harvest, the activity was always intermediate between the individual BMPs, demonstrating that the BMP co-expressed heterodimers yield higher activity than combinations of the individually expressed BMP homodimers.

### EXAMPLE 3 - STABLE EXPRESSION OF BMP HETERODIMERS

#### A. BMP-2/7

Based on the results of the transient assays in Example 2, stable cell lines were made that co-express BMP-2 and BMP-7.

A preferred stable cell line, 2E7E-10, was obtained as follows: Plasmid DNA (a 1:1 mixture of pBMP-7-EMC and pBMP-2-EMC, described in Example 1) is transfected into CHO cells by electroporation [Neuman et al, EMBO J., 1:841-845 (1982)].

Two days later, cells are switched to selective medium containing 10% dialyzed fetal bovine serum and lacking nucleosides. Colonies expressing DHFR are counted 10-14 days later. Individual colonies or pools of colonies are expanded and analyzed for expression of each heterodimer BMP component RNA and protein using standard procedures and are subsequently selected for amplification by growth in increasing concentrations of MTX. Stepwise selection of the preferred clone, termed 2E7E, is carried out up to a concentration of 0.5  $\mu$ M MTX.

The cell line is then subcloned and assayed for heterodimer 2/7 expression.

Procedures for such assay include Western blot analysis to detect the presence of the component DNA, protein analysis and SDS-PAGE analysis of metabolically labelled protein, W20 assay, and analysis for cartilage and/or bone formation activity using the ectopic rat bone formation assay of Example 9. The presently preferred clonally-derived cell line is identified as 2E7E-10. This cell line secretes BMP-2/7 heterodimer proteins into the media containing 0.5  $\mu$ M MTX.

The CHO cell line 2E7E-10 is grown in Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12, 1:1 (vol/vol), supplemented with 10% fetal bovine serum. When the cells are 80 to 100% confluent, the medium is replaced with serum-free DMEM/F-12. Medium is harvested every 24 hours for 4 days. For protein production and purification the cells are cultured serum-free.

While the co-expressing cell line 2E7E-10 preliminarily appears to make lower amounts of BMP protein than the BMP2-expressing cell line 2EG5 described in Example 2, preliminary evidence suggests that the specific activity of the presumptive heterodimer is at least 5-fold greater than BMP-2 homodimer (see Example 6).

To construct another heterodimer producing cell

line, the stable CHO cell line 7MB9, previously transfected with pBMP-7-pMT2, and which expresses BMP-7, is employed. 7MB9 may be amplified and selected to 2  $\mu$ M methotrexate resistance using the DHFR/MTX system. To generate a stable co-expressing cell line, cell line 7MB9 is transfected with the expression vector pBMP-2 $\Delta$ -EN (EMC-Neo) containing BMP-2 and the neomycin resistance gene from the Tn5 transposable element. The resulting transfected stable cell line was selected for both G-418 and MTX resistance. Individual clones were picked and analyzed for BMP expression, as described above.

It is anticipated that stable cell lines co-expressing other combinations of BMPs which show enhanced activity by transient coexpression will likewise yield greater activity upon stable expression.

B. BMP-2/6

Based on the results of the transient assays in Example 2, stable cell lines were made that co-express BMP-2 and BMP-6.

A preferred stable cell line, 12C07, was obtained as follows: Plasmid DNA (a 1:3 mixture of pBMP-6-EMC and pBMP-2-EMC, described in Example 1) is transfected into CHO cells by electroporation [Neuman et al, EMBO J., 1:841-845 (1982)].

Two days later, cells are switched to selective medium containing 10% dialyzed fetal bovine serum and lacking nucleosides. Colonies expressing DHFR are

counted 10-14 days later. Individual colonies or pools of colonies are expanded and analyzed for expression of each heterodimer BMP component RNA and protein using standard procedures and are subsequently selected for amplification by growth in increasing concentrations of MTX. Stepwise selection of the preferred clone, termed 12-C, is carried out up to a concentration of 2.0  $\mu$ M MTX. The cell line is then subcloned and assayed for heterodimer 2/6 expression.

Procedures for such assay include Western blot analysis to detect the presence of the component DNA, protein analysis and SDS-PAGE analysis of metabolically labelled protein, W20 assay, and analysis for cartilage and/or bone formation activity using the ectopic rat bone formation assay of Example 9. The presently preferred clonally-derived cell line is identified as 12C07. This cell line secretes BMP-2/6 heterodimer proteins into the media containing 2.0  $\mu$ M MTX.

The CHO cell line 12C07 is grown in Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12, 1:1 (vol/vol), supplemented with 10% fetal bovine serum. When the cells are 80 to 100% confluent, the medium is replaced with serum-free DMEM/F-12. Medium is harvested every 24 hours for 4 days. For protein production and purification the cells are cultured serum-free.

While the co-expressing cell line 12C07

preliminarily appears to make lower amounts of BMP protein than the BMP2-expressing cell line 2EG5 described in Example 2, preliminary evidence suggests that the specific activity of the presumptive heterodimer is at least 3-5-fold greater than BMP-2 homodimer (see Example 6).

To construct another heterodimer producing cell line, the stable CHO cell line 2EG5, previously transfected with pBMP-2-EMC, and which expresses BMP-2, is employed. 2EG5 may be amplified and selected to 2  $\mu$ M methotrexate resistance using the DHFR/MTX system. To generate a stable co-expressing cell line, cell line 2EG5 is transfected with the expression vector pBMP-6-ada (ada deaminase) containing BMP-6 and the ADA resistance gene. The resulting transfected stable cell line was selected for both DCF and MTX resistance. Individual clones are picked and analyzed for BMP expression, as described above.

It is anticipated that stable cell lines co-expressing other combinations of BMPs which show enhanced activity by transient coexpression will likewise yield greater activity upon stable expression.

#### EXAMPLE 4-PURIFICATION OF BMP2/7 AND BMP-2/6 HETERODIMER

The same purification procedure is used for BMP-2/6 heterodimer and BMP-2/7 heterodimer. Conditioned media from cultures of cell line 2E7E-10 or 12C07 containing

recombinantly produced BMP heterodimer 2/7V or 2/6, respectively, can be generated from either adherent or suspension cultures. For small to medium scale generation of coexpressed BMP, adherent cultures are seeded into roller bottles and allowed to grow to confluence in alpha-Minimal Eagles Medium [ $\alpha$ -MEM, Gibco, Grand Island, NY] containing 10% dialyzed heat-inactivated fetal calf serum [Hazleton, Denver, PA]. The media is then switched to a serum-free, albumin free, low protein medium based on a 50:50 mixture of Delbecco's Modified Eagle's medium and Hams F-12 medium, optionally supplemented with 100 micrograms/ml dextran sulfate. Four or five daily harvests are pooled, and used to purify the recombinant protein.

Conditioned medium from roller bottle cultures obtained as described above was thawed slowly at room temperature and pooled. The pH of the pooled medium was adjusted to pH 8.0 using 1 M Tris, pH 8.0. A column was poured containing Matrex Cellufine Sulfate [Amicon] and equilibrated in 50 mM Tris, pH 8.0.

Upon completion of loading of the medium, the column was washed with buffer containing 50 mM Tris, 0.4 M NaCl, pH 8.0 until the absorbance at 280 nm reached baseline. The column was then washed with 50 mM Tris, pH 8.0 to remove NaCl from the buffer. The resin was then washed with 50 mM Tris, 0.2 M NaCl, 4 M Urea, pH 8.0 until a peak had eluted. The column was then washed into

50 mM Tris, pH 8.0 to remove the urea.

The bound BMP-2/7 or BMP-2/6 was then eluted using 50 mM Tris, 0.5 M NaCl, 0.5 M Arginine, pH 8.0. The eluate was collected as a single pool and may be optionally stored frozen prior to further purification. This Cellufine Sulfate eluate was diluted with 14 volumes of 6M urea and the pH of the sample was then adjusted to 6.0. A hydroxyapatite-Ultrogel [IBF] column was poured and equilibrated with 80 mM potassium phosphate, 6M urea, pH 6.0.

After the completion of sample loading, the column was washed with 10 bed volumes of the equilibration buffer. Bound BMP-2/7 or BMP-2/6 heterodimers were eluted with 5 bed volumes of 100 mM potassium phosphate, 6M urea, pH 7.4. This eluate was loaded directly onto a Vydac C<sub>4</sub> reverse-phase HPLC column equilibrated in water - 0.1% TFA. BMP-2/7 or BMP-2/6 heterodimers were eluted with a gradient of 30-50% acetonitrile in water - 0.1% trifluoroacetic acid.

Fractions containing BMPs are identified by SDS-PAGE in the presence or absence of reductant. The identity of the BMPs with respect to the heterodimers vs. homodimers is determined by 2D-PAGE (+/- reductant). Fractions with heterodimers gave bands which reduce to two spots. Bands from homodimer fractions reduce to a single spot for each BMP species.

The BMP-2/6 heterodimer subunits are analyzed on a protein sequenator. BMP-2/6 heterodimers of the followig species are present: BMP-6 subunit beginning with amino acid #375 Ser-Ala-Ser-Ser in association with  
5 BMP-2 subunit beginning with amino acid #283 Gin-Ala-Lys or #249 Ser-Lev-His, though other less abundant species may be present.

It is contemplated that the same or substantially similar purification techniques may be employed for any  
10 recombinant BMP heterodimer of this invention. The hydroxyapatite-Ultrogel column may be unnecessary and that the purification scheme may be modified by loading the Cellufine Sulfate eluate directly onto the C<sub>4</sub> reverse-phase HPLC column without use of the former column for  
15 BMP2/7 or BMP-2/6 or the other heterodimers of this invention.

#### EXAMPLE 5 - PROTEIN CHARACTERIZATION

Total protein secreted from the co-expressing cell lines is analyzed after labelling with <sup>35</sup>S-methionine  
20 or by Western blot analysis using antibodies raised against both BMPs of the heterodimer, e.g., BMP-2 and BMP-7. Together with the alkaline phosphatase assays, the data indicates the presence of the heterodimer and the specific activity. The following specific details  
25 are directed towards data collected for the BMP-2/7 and BMP-2/6 heterodimers; however, by application of similar

methods to the other heterodimers described herein,  
similar results are expected.

A. <sup>35</sup>S-Met labelling

Cell lines derived by cotransfection of  
BMP2 $\Delta$ -EMC and BMP7 $\Delta$ -EMC expression vectors were pulsed  
with <sup>35</sup>S-methionine for 15 minutes, and chased for 6 hours  
in serum free media in the presence or absence of  
heparin. Total secreted protein was analyzed under  
reducing conditions by PAGE and fluorography. The  
results demonstrate that several cell lines secrete both  
BMP-2 and BMP-7 protein. There is a good correlation  
between the amount of alkaline phosphatase activity and  
the amount of coexpressed protein.

Several cell lines secrete less total BMP-  
2 and 7 than the BMP-2-only expressing cell line 2EG5,  
which produces 10  $\mu$ g/ml BMP-2. Cell line 2E7E-10  
(amplified at a level of 0.5mM MTX) secretes equal  
proportions of BMP-2 and BMP-7 at about the same overall  
level of expression as the cell line 2EG5. Cell line  
2E7E-10 produces the equivalent of 600 micrograms/ml of  
BMP-2 homodimer activity in one assay.

Total labelled protein was also analyzed on a  
two-dimensional non-reducing/reducing gel system to  
ascertain whether a heterodimer is made. Preliminary  
results demonstrate the presence of a unique spot in this  
gel system that is not found in either the BMP-2-only or  
BMP-7-only cell lines, suggesting the presence of 2/7

heterodimer. The same gel with purified material produced the same results (e.g., two unique spots on the gel) indicative of the presence of the 2/7 heterodimer. The homodimer of BMP2 produced distinct species on this gel system.

In contrast to the recombinant BMP-2/7 purification, BMP-2 homodimers are not detected during the BMP-2/6 preparation; however, significant amounts of BMP-6 homodimers are found. In addition, a significant amount of a -20 amino acid N-terminal truncated form of BMP-6 is found; this could be eliminated by the inclusion of protease inhibitors during cell culture. BMP-2/6 was found to elute two to three fractions later from C4 RP-HPLC than did BMP-2/7.

Amino acid sequencing indicates that the predominant BMP-2/7 heterodimer species comprises a mature BMP-2 subunit [amino acid #283(Gln)-#396(Arg)] and a mature subunit of BMP-7 [#293(Ser)-#431(His)]. BMP-2/6 heterodimer comprises the mature BMP-2 subunit (#283-396) and the mature BMP-6 subunit [#375(Ser)-#513(His)].

#### B. Immunoprecipitation coupled to Western blot analysis

Conditioned media from a BMP-2-only (2EG5), a BMP-7-only (7MB9), or the 2E7E-10 co-expressing cell line were subjected to immunoprecipitation with either a BMP-2 or BMP-7 antibody (both conventional

polyclonal antibodies raised in rabbits), then analyzed on Western blots probed with either an anti-BMP-2 or anti-BMP-7 antibody. The 2/7 heterodimer precipitates and is reactive on Western blots with both the BMP-2 and BMP-7 antibodies, while either BMP by itself reacts with its specific antibody, but not with the reciprocal antibody.

It has been demonstrated using this strategy that a protein in the co-expressing cell line that is precipitated by the anti-BMP-7 antibody W33 [Genetics Institute, Inc, Cambridge, Massachusetts] and reacts on a Western blot with the anti-BMP-2 antibody W12 or W10 [Genetics Institute, Inc.] is not present in the BMP-2 or 7-only expressing cell lines. This experiment indicates that this protein species is the heterodimeric protein. Conversely, precipitation with W12 and probing with W33 yielded similar results.

#### EXAMPLE 6 - SPECIFIC ACTIVITY OF HETERODIMERS

##### A. In vitro Assays

The specific activity of the BMP-2/7 or BMP-2/6 heterodimer and the BMP-2 homodimer secreted into growth medium of the stable cell lines 2E7E-10 and 2EG55, and 12C07 and 2EG5, respectively, were estimated as follows.

The amount of BMP protein in conditioned medium was measured by either Western blot analysis or by

analyzing protein secreted from <sup>35</sup>S-methionine labelled cells by PAGE and fluorography. The amount of activity produced by the same cell lines on W20 cells using either the alkaline phosphatase assay or osteocalcin-induction assay was then estimated. The specific activity of the BMP was calculated from the ratio of activity to protein secreted into the growth medium.

In one experiment 2E7E-10 and 2EG5 secreted similar amounts of total BMP proteins as determined by PAGE and fluorography. 2E7E-10 produced about 50-fold more alkaline phosphatase inducing activity than 2EG5, suggesting that the specific activity of the heterodimer is about 50-fold higher than the homodimer.

In another experiment the amount of BMP-2 secreted by 2EG5 was about 50% higher than BMP-2/7 secreted by 2E7E-10, however, 2E7E-10 produced about 10-fold more osteocalcin-inducing activity than 2EG5. From several different experiments of this type the specific activity of the BMP-2/7 heterodimer is estimated to be between 5 to 50 fold higher than the BMP-2 homodimer.

Figures 8 and 9 compare the activity of BMP-2 and BMP-2/7 in the W20 alkaline phosphatase and BGP (Bone Gla Protein, osteocalcin) assays. BMP-2/7 has greatly increased specific activity relative to BMP-2 (Figure 8). From Figure 8, approximately 1.3 ng/ml of BMP-2/7 was sufficient to induce 50% of the maximal alkaline phosphatase response in W-20 cells. A comparable value

for BMP-2 is difficult to calculate, since the alkaline phosphatase response did not maximize, but greater than 30 ng/ml is needed for a half-maximal response. BMP-2/7 thus has a 20 to 30-fold higher specific activity than BMP-2 in the W-20 assay.

As seen in Figure 9, BMP-2/7 was also a more effective stimulator of BGP (bone gla protein, osteocalcin) production than BMP-2 in this experiment. Treating W-20-17 cells with BMP-2/7 for four days resulted in a maximal BGP response with 62 ng/ml, and 11 ng/ml elicits 50% of the maximal BGP response. In contrast, maximal stimulation of BGP synthesis by BMP-2 was not seen with doses up to 468 ng/ml of protein. The minimal dose of BMP-2/7 needed to elicit a BGP response by W-20-17 cells was 3.9 ng/ml, about seven-fold less than the 29 ng/ml required of BMP-2. These results were consistent with the data obtained in the W-20-17 alkaline phosphatase assays for BMP-2 and BMP-2/7.

Preliminary analysis indicates that BMP-2/6 has a specific activity in vitro similar to that of BMP-2/7. The potencies of BMP-2 and BMP-2/6 on induction of alkaline phosphatase production in W-20 is compared, as shown in Figure 12, BMP-2/6 has a higher specific activity than BMP-2 in this assay system. This data is in good agreement with data obtained from the *in vivo* assay of BMP-2 and BMP-2/6).

**B. In Vivo Assay****(i) BMP-2/7**

The purified BMP-2/7 and BMP-2 were tested in the rat ectopic bone formation assay. A series of different amounts of BMP-2/7 or BMP-2 were implanted in triplicate in rats. After 5 and 10 days, the implants were removed and examined histologically for the presence of bone and cartilage. The histological scores for the amounts of new cartilage and bone formed are summarized in Table A.

Table A

		5 Day Implants		10 Day Implants	
		BMP-2/7	BMP-2	BMP-2/7	BMP-2
0.04	C	$\pm - \pm$	- - -	$\pm - \pm$	- - -
	B	- - -	- - -	$\pm - \pm$	- - -
0.02	C	$\pm 1 \pm$	- - -	2 1 2	- $\pm \pm$
	B	- - -	- - -	1 $\pm 1$	- $\pm -$
1.0	C	1 $\pm \pm$	$\pm \pm \pm$	2 2 2	1 1 $\pm$
	B	- - -	- - -	2 3 3	1 1 $\pm$
5.0	C	2 2 1	1 $\pm 1$	1 1 2	1 2 1
	B	$\pm - 1$	- - -	4 4 3	2 3 2
25.0	C			$\pm \pm 2$	2 2 2
	B			4 4 3	3 3 3

The amount of BMP-2/7 required to induce cartilage and bone in the rat ectopic assay is lower than that of BMP-2. Histologically, the appearance of cartilage and bone induced by BMP-2/7 and BMP-2 are identical.

5 (ii) BMP-2/6

The *in vivo* activity of BMP-2/6 was compared with that of BMP-2 by implantation of various amounts of each BMP for ten days in the rat ectopic bone formation assay. The results of this study (Table B, Figure 13) indicate that BMP-2/6, similar to BMP-2/7, has increased *in vivo* activity relative to BMP-2. The specific activities of BMP-2, BMP-6, and BMP-2/6 are compared in the ectopic bone formation assay ten days after the proteins are implanted. The results of these experiments are shown in Table C and Figure 14. BMP-2/6 is a more potent inducer of bone formation than either BMP-2 or BMP-6. The amount

of bone formation observed with BMP-2/6 was comparable to that observed with equivalent doses of BMP-2/7. The appearance of BMP-2/6 implants is quite similar to implants containing BMP-2 or BMP-2/7.

5

**Table B**  
Histological scores of Implants of BMP 2/6 and BMP-2 in rat ectopic assay (10 day implants).

	BMP ( $\mu$ g)	C/B	BMP-2/6	BMP-2
	0.04	C	- ± -	- - -
		B	- - -	- - -
10	0.20	C	1 1 ±	- - -
		B	± ± ±	- - -
	1.0	C	1 3 3	1 1 ±
		B	1 2 2	1 1 ±
	5.0	C	2 2 2	1 2 2
		B	2 3 3	2 2 2
	25.	C	1 1 1	2 2 1
		B	3 3 3	3 3 3

15

**Table C**  
Histological scores of implants of BMP-2, BMP-6, and BMP-2/6 in rat ectopic assay (10 day implants).

	BMP ( $\mu$ g)	C/B	BMP-2	BMP-6	BMP-2/6
	0.04	C	- - -	- - -	- - ±
		B	- - -	- - -	- - ±
	0.20	C	- - 2	- - -	1 2 2
		B	- - 1	- - -	2 2 2
20	1.0	C	- ± ±	2 1 1	1 1 1
		B	- ± ±	1 ± ±	3 3 2
	5.0	C	2 2 1	3 1 3	± ± 1
		B	1 1 1	2 ± 1	4 5 4
	25.	C	± ± ±	± ± ±	± ± ±
		B	5 4 5	4 4 5	4 5 3

#### EXAMPLE 7 - EXPRESSION OF BMP DIMER IN E. COLI

A biologically active, homodimeric BMP-2 was expressed in E. coli using the techniques described in

25

European Patent Application 433,255 with minor modifications. Other methods disclosed in the above-referenced European patent application may also be employed to produce heterodimers of the present invention from E. coli. Application of these methods to the heterodimers of this invention is anticipated to produce active BMP heterodimeric proteins from E. coli.

A. BMP-2 Expression Vector

An expression plasmid pALBP2-781 (Figure 7) (SEQ ID NO: 13) was constructed containing the mature portion of the BMP-2 (SEQ ID NO: 14) gene and other sequences which are described in detail below. This plasmid directed the accumulation of 5-10% of the total cell protein as BMP-2 in an E. coli host strain, GI724, described below.

Plasmid pALBP2-781 contains the following principal features. Nucleotides 1-2060 contain DNA sequences originating from the plasmid pUC-18 [Norrrander et al, Gene, 26:101-106 (1983)] including sequences containing the gene for  $\beta$ -lactamase which confers resistance to the antibiotic ampicillin in host E. coli strains, and a colE1-derived origin of replication. Nucleotides 2061-2221 contain DNA sequences for the major leftward promoter (pL) of bacteriophage  $\lambda$  [Sanger et al, J. Mol. Biol., 162:729-773 (1982)], including three operator sequences, O<sub>L</sub>1, O<sub>L</sub>2 and O<sub>L</sub>3. The operators are the binding sites for  $\lambda$ cI repressor protein,

intracellular levels of which control the amount of transcription initiation from pL. Nucleotides 2222-2723 contain a strong ribosome binding sequence included on a sequence derived from nucleotides 35566 to 35472 and 38137 to 38361 from bacteriophage lambda as described in Sanger et al, J. Mol. Biol., 162:729-773 (1982). Nucleotides 2724-3133 contain a DNA sequence encoding mature BMP-2 protein with an additional 62 nucleotides of 3'-untranslated sequence.

10 Nucleotides 3134-3149 provide a "Linker" DNA sequence containing restriction endonuclease sites. Nucleotides 3150-3218 provide a transcription termination sequence based on that of the E. coli aspA gene [Takagi et al, Nucl. Acids Res., 13:2063-2074 (1985)].

15 Nucleotides 3219-3623 are DNA sequences derived from pUC-18.

As described below, when cultured under the appropriate conditions in a suitable E. coli host strain, pALBP2-781 can direct the production of high levels (approximately 10% of the total cellular protein) of BMP-2 protein.

pALBP2-781 was transformed into the E. coli host strain GI724 (F, lacI<sup>q</sup>, lacP<sup>L8</sup>, ampC::λcI<sup>+</sup>) by the procedure of Dagert and Ehrlich, Gene, 6:23 (1979). [The untransformed host strain E. coli GI724 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland on January 31, 1991 under ATCC

No. 55151 for patent purposes pursuant to applicable laws and regulations.] Transformants were selected on 1.5% w/v agar plates containing IMC medium, which is composed of M9 medium [Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory, New York (1972)] supplemented with 0.5% w/v glucose, 0.2% w/v casamino acids and 100 µg/ml ampicillin.

GI724 contains a copy of the wild-type  $\lambda$ C<sub>I</sub> repressor gene stably integrated into the chromosome at the ampC locus, where it has been placed under the transcriptional control of Salmonella typhimurium trp promoter/operator sequences. In GI724,  $\lambda$ C<sub>I</sub> protein is made only during growth in tryptophan-free media, such as minimal media or a minimal medium supplemented with casamino acids such as IMC, described above. Addition of tryptophan to a culture of GI724 will repress the trp promoter and turn off synthesis of  $\lambda$ C<sub>I</sub>, gradually causing the induction of transcription from pL promoters if they are present in the cell.

GI724 transformed with pALBP2-781 was grown at 37°C to an A<sub>550</sub> of 0.5 (Absorbance at 550 nm) in IMC medium. Tryptophan was added to a final concentration of 100 µg/ml and the culture incubated for a further 4 hours. During this time BMP-2 protein accumulated to approximately 10% of the total cell protein, all in the "inclusion body" fraction.

BMP-2 is recovered in a non-soluble,

monomeric form as follows. Cell disruption and recovery is performed at 4°C. Approximately 9 g of the wet fermented E. coli GI724/pALBP2-781 cells are suspended in 30 mL of 0.1 M Tris/HCl, 10 mM EDTA, 1 mM phenyl methyl sulphonyl fluoride (PMSF), pH 8.3 (disruption buffer). The cells are passed four times through a cell disrupter and the volume is brought to 100 mL with the disruption buffer. The suspension is centrifuged for 20 min. (15,000 x g). The pellet obtained is suspended in 50 mL disruption buffer containing 1 M NaCl and centrifuged for 10 min. as above. The pellet is suspended in 50 mL disruption buffer containing 1% Triton X-100 (Pierce) and again centrifuged for 10 min. as above. The washed pellet is then suspended in 25 mL of 20 mM Tris/HCl, 1 mM EDTA, 1 mM PMSF, 1% DTT, pH 8.3 and homogenized in a glass homogenizer. The resulting suspension contains crude monomeric BMP-2 in a non-soluble form.

Ten mL of the BMP-2 suspension, obtained as described above, are acidified with 10% acetic acid to pH 2.5 and centrifuged in an Eppendorf centrifuge for 10 min. at room temperature. The supernatant is chromatographed. Chromatography was performed on a Sephacryl S-100 HR column (Pharmacia, 2.6 x 83 cm) in 1% acetic acid at a flow rate of 1.4 mL/minute. Fractions containing monomeric, BMP-2 are pooled. This material is used to generate biologically active, homodimer BMP-2.

Biologically active, homodimeric BMP-2 can

be generated from the monomeric BMP-2 obtained following solubilization and purification, described above, as follows.

0.1, 0.5 or 2.5 mg of the BMP-2 is dissolved at a concentration of 20, 100 or 500  $\mu\text{g/mL}$ , respectively, in 50 mM Tris/HCl, pH 8.0, 1 M NaCl, 5 mM EDTA, 2 mM reduced glutathione, 1 mM oxidized glutathione and 33 mM CHAPS [Calbiochem]. After 4 days at 4°C or 23°C, the mixture is diluted 5 to 10 fold with 0.1% TFA.

Purification of biologically active BMP-2 is achieved by subjecting the diluted mixture to reverse phase HPLC on a a Vydac C4 214TP54 column (25 x .46 cm) [The NEST Group, USA] at a flow rate of 1 ml/minute. Buffer A is 0.1% TFA. Buffer B is 90% acetonitrile, and 0.1% TFA. The linear gradient was 0 to 5 minutes at 20% Buffer B; 5 to 10 minutes at 20 to 30 % Buffer B; 10 to 40 minutes at 30 to 60% Buffer B; and 40 to 50 minutes at 60 to 100% Buffer B. Homodimeric BMP-2 is eluted and collected from the HPLC column.

The HPLC fractions are lyophilized to dryness, redissolved in sample buffer (1.5 M Tris-HCl, pH 8.45, 12% glycerol, 4% SDS, .0075% Serva Blue G, .0025% Phenol Red, with or without 100 mM dithiothreitol) and heated for five minutes at 95°C. The running buffer is 100 mM Tris, 100 mM tricine (16% tricine gel) [Novex], 0.1% SDS at pH 8.3. The SDS-PAGE gel is run at 125 volts for 2.5 hours.

The gel is stained for one hour with 200 ml of 0.5% Coomassie Brilliant Blue R-250, 25% isopropanol, 10% acetic acid, heated to 60°C. The gel is then destained with 10% acetic acid, 10% isopropanol until the background is clear.

The reduced material ran at approximately 13kD; the non-reduced material ran at approximately 30 kD, which is indicative of the BMP-2 dimer. This material was later active in the W20 assay of Example 8.

10           B. BMP-7 Expression Vector

For high level expression of BMP-7 a plasmid pALBMP7-981 was constructed. pALBMP7-981 is identical to plasmid pALBP2-781 with two exceptions: the BMP-2 gene (residues 2724-3133 of pALBP2-781) is replaced by the mature portion of the BMP-7 gene, deleted for sequenced encoding the first seven residues of the mature BMP-7 protein sequence:

ATGTCTCATAATC GTTCTAAAAC TCCAAAAAAT CAAGAAGCTC TCGGTATGGC

CAACGTGGCA GAGAACAGCA GCAGCGACCA GAGGCAGGCC TGTAAGAAGC  
 ACGAGCTGTA TGTCAGCTTC CGAGACCTGG GCTGGCAGGA CTGGATCATC  
 GCGCCTGAAG GCTACGCCGC CTACTACTGT GAGGGGGAGT GTGCCTTCCC  
 5 TCTGAACTCC TACATGAACG CCACCAACCA CGCCATCGTG CAGACGCTGG  
 TCCACTTCAT CAACCCGGAA ACGGTGCCCA AGCCCTGCTG TCGCCCCACG  
 CAGCTCAATG CCATCTCCGT CCTCTACTTC GATGACAGCT CCAACGTCAT  
 CCTGAAGAAA TACAGAAACA TGGTGGTCCG GGCCTGTGGC TGCCACTAGC  
 TCCTCCGAGA ATTCAGACCC TTTGGGGCCA AGTTTTTCTG GATCCT

10 and the ribosome binding site found between residues  
 2707 and 2723 in pALBP2-781 is replaced by a different  
 ribosome binding site, based on that found preceding the  
 T7 phage gene 10, of sequence 5'-CAAGAAGGAGATATACAT-3'.  
 The host strain and growth conditions used for the  
 15 production of BMP-7 were as described for BMP-2.

### C. BMP-3 Expression Vector

For high level expression of BMP-3 a  
 plasmid pALB3-782 was constructed. This plasmid is  
 identical to plasmid pALBP2-781, except that the BMP-2  
 20 gene (residues 2724-3133 of pALBP2-781) is replaced by a  
 gene encoding a form of mature BMP-3. The sequence of  
 this BMP-3 gene is:

80

ATGCGTAAAC AATGGATTGA ACCACGTAAC TGTGCTCGTC GTTATCTGAA  
AGTAGACTTT GCAGATATTG GCTGGAGTGA ATGGATTATC TCCCCCAAGT  
CCTTTGATGC CTATTATTGC TCTGGAGCAT GCCAGTTCCC CATGCCAAAG  
TCTTTGAAGC CATCAAATCA TGCTACCATC CAGAGTATAG TGAGAGCTGT  
5 GGGGGTCGTT CCTGGGATTC CTGAGCCTTG CTGTGTACCA GAAAAGATGT  
CCTCACTCAG TATTTTATTC TTTGATGAAA ATAAGAATGT AGTGCTTAAA  
GTATACCCTA ACATGACAGT AGAGTCTTGC GCTTGCAGAT AACCTGGCAA  
AGAACTCATT TGAATGCTTA ATTCAAT

The host strain and growth conditions used for the  
10 production of BMP-3 were as described for BMP-2.

D. Expression of a BMP-2/7 Heterodimer in E. coli

Denatured and purified E. coli BMP-2 and BMP-7  
monomers were isolated from E. coli inclusion body  
15 pellets by acidification and gel filtration as previously  
as previously described above. 125 ug of each BMP in 1%  
acetic acid were mixed and taken to dryness in a speed  
vac. The material was resuspended in 2.5 ml 50 mM Tris,  
1.0 NaCl, 5 mM EDTA, 33 mM CHAPS, 2 mM glutathione  
20 (reduced), 1 mM glutathione (oxidized), pH 8.0. The  
sample was incubated at 23 C for one week.

The BMP-2/7 heterodimer was isolated by  
HPLC on a 25 x 0.46 cm Vydac C4 column. The sample was  
centrifuged in a microfuge for 5 minutes, and the  
25 supernatant was diluted with 22.5 ml 0.1% TFA.

A buffer : 0.1% TFA

B buffer : 0.1% TFA, 95% acetonitrile

81

1.0 ml/minute

0-5' 20% B

5-10' 20-30% B

10-90' 30-50% B

5 90-100' 50-100% B

By SDS-PAGE analysis, the BMP-2/7 heterodimer eluted at about 23'.

Figure 10 is a comparison of the W-20 activity of E. coli BMP-2 and BMP-2/7 heterodimer, indicating greater activity of the heterodimer.

10

F. Expression of BMP-2/3 Heterodimer in E. coli

BMP-2 and BMP-3 monomers were isolated as follows: to 1.0 g of frozen harvested cells expressing either BMP-2 or BMP-3 was added 3.3 ml of 100 mM Tris, 10 mM EDTA, pH 8.3. The cells were resuspended by vortexing vigorously. 33 ul of 100 mM PMSF in isopropanol was added and the cells lysed by one pass through a French pressure cell. The lysate was centrifuged in a microfuge for 20 minutes at 4 C. The supernatant was discarded. The inclusion body pellet was taken up in 8.0 M guanidine hydrochloride, 0.25 M OTT, 0.5 M Tris, 5 mM EDTA, pH 8.5, and heated at 37 C for one hour.

20

The reduced and denatured BMP monomers were isolated by HPLC on a Supelco C4 guard column as follows:

25

A buffer : 0.1% TFA

B buffer : 0.1% TFA, 95% acetonitrile

82

1.0 ml/minute

0-5' 1% B

5-40' 1-70% B

40-45' 70-100% B

5 Monomeric BMP eluted at 28-30'. Protein concentration was estimated by A280 and the appropriate extinction coefficient.

10 10 ug of BMP-2 and BMP-3 were combined and taken to dryness in a speed vac. To this was added 50 ul of 50 mM Tris, 1.0 M NaCl, 5 mM EDTA, 33 mM CHAPS, 2 mM reduced glutathione, 1 mM oxidized glutathione, pH 8.5. The sample was incubated at 23 for 3 days. The sample was analyzed by SDS-PAGE on a 16% tricine gel under reducing and nonreducing conditions. The BMP-2/3 heterodimer  
15 migrated at about 35 kd nonreduced, and reduced to BMP-2 monomer at about 13 kd and BMP-3 monomer at about 21 kd.

BMP-2/3 heterodimer produced in *E. coli* is tested for *in vivo* activity. (20 µg) at (ten days) is utilized to compare the *in vivo* activity of BMP-2/3 to  
20 BMP-2. BMP-2/3 implants showed no cartilage or bone forming activity, while the BMP-2 control implants showed the predicted amounts of bone and cartilage formation. The *in vivo* data obtained with BMP-2/3 is consistent with the *in vitro* data from the W-20 assay.

EXAMPLE 8 - W-20 BIOASSAYSA. Description of W-20 cells

Use of the W-20 bone marrow stromal cells as an indicator cell line is based upon the conversion of these cells to osteoblast-like cells after treatment with BMP-2 [R. S. Thies et al, "Bone Morphogenetic Protein alters W-20 stromal cell differentiation in vitro", Journal of Bone and Mineral Research, 5(2):305 (1990); and R. S. Thies et al, "Recombinant Human Bone Morphogenetic Protein 2 Induces Osteoblastic Differentiation in W-20-17 Stromal Cells", Endocrinology, in press (1992)]. Specifically, W-20 cells are a clonal bone marrow stromal cell line derived from adult mice by researchers in the laboratory of Dr. D. Nathan, Children's Hospital, Boston, MA. BMP-2 treatment of W-20 cells results in (1) increased alkaline phosphatase production, (2) induction of PTH stimulated cAMP, and (3) induction of osteocalcin synthesis by the cells. While (1) and (2) represent characteristics associated with the osteoblast phenotype, the ability to synthesize osteocalcin is a phenotypic property only displayed by mature osteoblasts. Furthermore, to date we have observed conversion of W-20 stromal cells to osteoblast-like cells only upon treatment with BMPs. In this manner, the in vitro activities displayed by BMP treated W-20 cells correlate with the in vivo bone forming activity known for BMPs.

Below two in vitro assays useful in comparison of BMP activities of novel osteoinductive molecules are described.

B. W-20 Alkaline Phosphatase Assay Protocol

5           W-20 cells are plated into 96 well tissue culture plates at a density of 10,000 cells per well in 200  $\mu$ l of media (DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 100 U/ml + 100  $\mu$ g/ml streptomycin. The cells are allowed to attach overnight  
10           in a 95% air, 5% CO<sub>2</sub> incubator at 37°C.

          The 200  $\mu$ l of media is removed from each well with a multichannel pipettor and replaced with an equal volume of test sample delivered in DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 1%  
15           penicillin-streptomycin. Test substances are assayed in triplicate.

          The test samples and standards are allowed a 24 hour incubation period with the W-20 indicator cells. After the 24 hours, plates are removed from the  
20           37°C incubator and the test media are removed from the cells.

          The W-20 cell layers are washed 3 times with 200  $\mu$ l per well of calcium/magnesium free phosphate buffered saline and these washes are discarded.

25           50  $\mu$ l of glass distilled water is added to each well and the assay plates are then placed on a dry ice/ethanol bath for quick freezing. Once frozen, the

85

assay plates are removed from the dry ice/ethanol bath and thawed at 37°C. This step is repeated 2 more times for a total of 3 freeze-thaw procedures. Once complete, the membrane bound alkaline phosphatase is available for measurement.

50 µl of assay mix (50 mM glycine, 0.05% Triton X-100, 4 mM MgCl<sub>2</sub>, 5 mM p-nitrophenol phosphate, pH = 10.3) is added to each assay well and the assay plates are then incubated for 30 minutes at 37°C in a shaking waterbath at 60 oscillations per minute.

At the end of the 30 minute incubation, the reaction is stopped by adding 100 µl of 0.2 N NaOH to each well and placing the assay plates on ice.

The spectrophotometric absorbance for each well is read at a wavelength of 405 nanometers. These values are then compared to known standards to give an estimate of the alkaline phosphatase activity in each sample. For example, using known amounts of p-nitrophenol phosphate, absorbance values are generated. This is shown in Table I.

Table I

Absorbance Values for Known Standards  
of P-Nitrophenol Phosphate

<u>P-nitrophenol phosphate umoles</u>	<u>Mean absorbance (405 nm)</u>
0.000	0
0.006	0.261 +/- .024
0.012	0.521 +/- .031
0.018	0.797 +/- .063

86

0.024  
0.030

1.074 +/- .061  
1.305 +/- .083

5 Absorbance values for known amounts of BMP-2 can be determined and converted to  $\mu$ moles of p-nitrophenol phosphate cleaved per unit time as shown in Table II.

Table II

10 Alkaline Phosphatase Values for W-20 Cells Treating with BMP-2

	BMP-2 concentration ng/ml	Absorbance Reading 405 nmeters	umoles substrate per hour
	0	0.645	0.024
15	1.56	0.696	0.026
	3.12	0.765	0.029
	6.25	0.923	0.036
	12.50	1.121	0.044
	25.0	1.457	0.058
20	50.0	1.662	0.067
	100.0	1.977	0.080

25 These values are then used to compare the activities of known amounts of BMP heterodimers to BMP-2 homodimer.

### C. Osteocalcin RIA Protocol

30 W-20 cells are plated at  $10^6$  cells per well in 24 well multiwell tissue culture dishes in 2 mls of DME containing 10% heat inactivated fetal calf serum, 2 mM glutamine. The cells are allowed to attach overnight in an atmosphere of 95% air 5% CO<sub>2</sub> at 37°C.

The next day the medium is changed to DME

containing 10% fetal calf serum, 2 mM glutamine and the test substance in a total volume of 2 ml. Each test substance is administered to triplicate wells. The test substances are incubated with the W-20 cells for a total of 96 hours with replacement at 48 hours by the same test medias.

At the end of 96 hours, 50  $\mu$ l of the test media is removed from each well and assayed for osteocalcin production using a radioimmunoassay for mouse osteocalcin. The details of the assay are described in the kit manufactured by Biomedical Technologies Inc., 378 Page Street, Stoughton, MA 02072. Reagents for the assay are found as product numbers BT-431 (mouse osteocalcin standard), BT-432 (Goat anti-mouse Osteocalcin), BT-431R (iodinated mouse osteocalcin), BT-415 (normal goat serum) and BT-414 (donkey anti goat IgG). The RIA for osteocalcin synthesized by W-20 cells in response to BMP treatment is carried out as described in the protocol provided by the manufacturer.

The values obtained for the test samples are compared to values for known standards of mouse osteocalcin and to the amount of osteocalcin produced by W-20 cells in response to challenge with known amounts of BMP-2. The values for BMP-2 induced osteocalcin synthesis by W-20 cells is shown in Table III.

Table III

## Osteocalcin Synthesis by W-20 Cells

	<u>BMP-2 Concentration ng/ml</u>	<u>Osteocalcin Synthesis ng/well</u>
5	0	0.8
	2	0.9
	4	0.8
	8	2.2
	16	2.7
10	31	3.2
	62	5.1
	125	6.5
	250	8.2
	500	9.4
15	1000	10.0

EXAMPLE 9 - ROSEN MODIFIED SAMPATH-REDDI ASSAY

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. USA, 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of BMP proteins. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved in 0.1% TFA, and the resulting solution added to 20 mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are

implanted subcutaneously in the abdominal thoracic area of 21-49 ay old male Long Evans rats. The implants are removed after 7-14 days. Half of each implant is used for alkaline phosphatase analysis [see, A. H. Reddi et al, Proc. Natl. Acad. Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. 1  $\mu$ m glycolmethacrylate sections are stained with Von Kossa and acid fuschin to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2, and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone.

The heterodimeric BMP proteins of this invention may be assessed for activity on this assay.

Numerous modifications and variations in practice of this invention are expected to occur to those skilled in the art. Such modifications and variations are encompassed within the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Israel, David  
Wolfman, Neil M.
- (ii) TITLE OF INVENTION: Recombinant Bone Morphogenetic Protein  
Heterodimers, Compositions and Methods of Use.
- (iii) NUMBER OF SEQUENCES: 30
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Legal Affairs, Genetics Institute, Inc.
  - (B) STREET: 87 CambridgePark Drive
  - (C) CITY: Cambridge
  - (D) STATE: MA
  - (E) COUNTRY: USA
  - (F) ZIP: 02140-2387
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Tape
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Kapinos, Ellen J.
  - (B) REGISTRATION NUMBER: 32,245
  - (C) REFERENCE/DOCKET NUMBER: GI-5192B
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 617-876-1170
  - (B) TELEFAX: 617-876-5851

## !) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1607 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 356..1543

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT	60
AACTTGCGCA CCCCACTTTG CGCCGGTGCC TTTGCCCCAG CGGAGCCTGC TTCGCCATCT	120
CCGAGCCCCA CCGCCCCTCC ACTCCTCGGC CTTGCCCCGAC ACTGAGACGC TGTTCCCAGC	180
GTGAAAAGAG AGACTGCGCG GCCGGCACCC GGGAGAAGGA GGAGGCAAAG AAAAGGAACG	240
GACATTCCGGT CCTTGCGCCA GGTCCTTTGA CCAGAGTTTT TCCATGTGGA CGCTCTTTCA	300
ATGGACGTGT CCCC GCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT CGACC ATG Met 1	358
GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC CTC Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val Leu 5 10 15	406
CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe 20 25 30	454
GCG GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC Ala Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val 35 40 45	502
CTG AGC GAG TTC GAG TTG CGG CTG CTC AGC ATG TTC GGC CTG AAA CAG Leu Ser Glu Phe Glu Leu Arg Leu Leu Ser Met Phe Gly Leu Lys Gln 50 55 60 65	550
AGA CCC ACC CCC AGC AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC Arg Pro Thr Pro Ser Arg Asp Ala Val Val Pro Pro Tyr Met Leu Asp 70 75 80	598
CTG TAT CGC AGG CAC TCA GGT CAG CCG GGC TCA CCC GCC CCA GAC CAC Leu Tyr Arg Arg His Ser Gly Gln Pro Gly Ser Pro Ala Pro Asp His 85 90 95	646
CGG TTG GAG AGG GCA GCC AGC CGA GCC AAC ACT GTG CGC AGC TTC CAC Arg Leu Arg Arg Ala Ala Ser Arg Ala Asn Thr Val Arg Ser Phe His 100 105 110	694
CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG AGT GGG AAA ACA ACC His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr Ser Gly Lys Thr Thr 115 120 125	742
CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG GAG TTT ATC Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu Glu Phe Ile 130 135 140 145	790
ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT TTA Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln Met Gln Asp Ala Leu 150 155 160	838
GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile 165 170 175	886
AAA CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGA CTT TTG GAC Lys Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu Asp	934

92															
180				185				190							
ACC	AGG	TTG	GTG	AAT	CAG	AAT	GCA	AGC	AGG	TGG	GAA	ACT	TTT	GAT	GTC
Thr	Arg	Leu	Val	Asn	Gln	Asn	Ala	Ser	Arg	Trp	Glu	Thr	Phe	Asp	Val
195						200					205				982
ACC	CCC	GCT	GTG	ATG	CGG	TGG	ACT	GCA	CAG	GGA	CAC	GCC	AAC	CAT	GGA
Thr	Pro	Ala	Val	Met	Arg	Trp	Thr	Ala	Gln	Gly	His	Ala	Asn	His	Gly
210					215					220					1030
TTC	GTG	GTG	GAA	GTG	GCC	CAC	TTG	GAG	GAG	AAA	CAA	GGT	GTC	TCC	AAG
Phe	Val	Val	Glu	Val	Ala	His	Leu	Glu	Glu	Lys	Gln	Gly	Val	Ser	Lys
				230					235					240	1078
AGA	CAT	GTT	AGG	ATA	AGC	AGG	TCT	TTG	CAC	CAA	GAT	GAA	CAC	AGC	TGG
Arg	His	Val	Arg	Ile	Ser	Arg	Ser	Leu	His	Gln	Asp	Glu	His	Ser	Trp
			245					250					255		1126
TCA	CAG	ATA	AGG	CCA	TTG	CTA	GTA	ACT	TTT	GGC	CAT	GAT	GGA	AAA	GGG
Ser	Gln	Ile	Arg	Pro	Leu	Leu	Val	Thr	Phe	Gly	His	Asp	Gly	Lys	Gly
			260				265					270			1174
CAT	CCT	CTC	CAC	AAA	AGA	GAA	AAA	CGT	CAA	GCC	AAA	CAC	AAA	CAG	CGG
His	Pro	Leu	His	Lys	Arg	Glu	Lys	Arg	Gln	Ala	Lys	His	Lys	Gln	Arg
	275					280					285				1222
AAA	CGC	CTT	AAG	TCC	AGC	TGT	AAG	AGA	CAC	CCT	TTG	TAC	GTG	GAC	TTC
Lys	Arg	Leu	Lys	Ser	Ser	Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	Asp	Phe
290						295				300					1270
AGT	GAC	GTG	GGG	TGG	AAT	GAC	TGG	ATT	GTG	GCT	CCC	CCG	GGG	TAT	CAC
Ser	Asp	Val	Gly	Trp	Asn	Asp	Trp	Ile	Val	Ala	Pro	Pro	Gly	Tyr	His
				310					315					320	1318
GCC	TTT	TAC	TGC	CAC	GGA	GAA	TGC	CCT	TTT	CCT	CTG	GCT	GAT	CAT	CTG
Ala	Phe	Tyr	Cys	His	Gly	Glu	Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu
			325					330					335		1366
AAC	TCC	ACT	AAT	CAT	GCC	ATT	GTT	CAG	ACG	TTG	GTC	AAC	TCT	GTT	AAC
Asn	Ser	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	Asn	Ser	Val	Asn
			340				345					350			1414
TCT	AAG	ATT	CCT	AAG	GCA	TGC	TGT	GTC	CCG	ACA	GAA	CTC	AGT	GCT	ATC
Ser	Lys	Ile	Pro	Lys	Ala	Cys	Cys	Val	Pro	Thr	Glu	Leu	Ser	Ala	Ile
	355					360					365				1462
TCG	ATG	CTG	TAC	CTT	GAC	GAG	AAT	GAA	AAG	GTT	GTA	TTA	AAG	AAC	TAT
Ser	Met	Leu	Tyr	Leu	Asp	Glu	Asn	Glu	Lys	Val	Val	Leu	Lys	Asn	Tyr
370					375					380					1510
CAG	GAC	ATG	GTT	GTG	GAG	GGT	TGT	GGG	TGT	CGC	TAGTACAGCA AAATTAAATA				1563
Gln	Asp	Met	Val	Val	Glu	Gly	Cys	Gly	Cys	Arg					
				390					395						
CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA AAAA															

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

93

(A) LENGTH: 396 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
 1           5           10           15
Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys
          20           25           30
Phe Ala Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu
          35           40           45
Val Leu Ser Glu Phe Glu Leu Arg Leu Leu Ser Met Phe Gly Leu Lys
          50           55           60
Gln Arg Pro Thr Pro Ser Arg Asp Ala Val Val Pro Pro Tyr Met Leu
          65           70           75           80
Asp Leu Tyr Arg Arg His Ser Gly Gln Pro Gly Ser Pro Ala Pro Asp
          85           90           95
His Arg Leu Glu Arg Ala Ala Ser Arg Ala Asn Thr Val Arg Ser Phe
          100          105          110
His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr Ser Gly Lys Thr
          115          120          125
Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu Glu Phe
          130          135          140
Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln Met Gln Asp Ala
          145          150          155          160
Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile
          165          170          175
Ile Lys Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu
          180          185          190
Asp Thr Arg Leu Val Asn Gln Asn Ala Ser Arg Trp Glu Thr Phe Asp
          195          200          205
Val Thr Pro Ala Val Met Arg Trp Thr Ala Gln Gly His Ala Asn His
          210          215          220
Gly Phe Val Val Glu Val Ala His Leu Glu Glu Lys Gln Gly Val Ser
          225          230          235          240
Lys Arg His Val Arg Ile Ser Arg Ser Leu His Gln Asp Glu His Ser
          245          250          255
Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys
          260          265          270
Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln

```

94

275	280	285
Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp 290 295 300		
Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr 305 310 315 320		
His Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His 325 330 335		
Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val 340 345 350		
Asn Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala 355 360 365		
Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn 370 375 380		
Tyr Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg 385 390 395		

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1954 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 403..1626

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA	60
GGTGAGTGTG GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG	120
AGTATCTAGC TTGTCTCCCC GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC	180
ACAGTCCCCG GCCCTCGCCC AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG	240
CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC	300
GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG CTGTCAAGAA	360
TCATGGACTG TTATTATATG CCTTGTTTTT TGTCAAGACA CC ATG ATT CCT GGT	414
Met Ile Pro Gly	
1	
AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC	462
Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly	
5 10 15 20	

95

GCG AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala	510
25 30 35	
GAG ATT CAG GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu	558
40 45 50	
CTC CTG CGG GAC TTC GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met Phe Gly Leu Arg	606
55 60 65	
CGC CGC CCG CAG CCT AGC AAG AGT GCC GTC ATT CCG GAC TAC ATG CGG Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro Asp Tyr Met Arg	654
70 75 80	
GAT CTT TAC CGG CTT CAG TCT GGG GAG GAG GAG GAA GAG CAG ATC CAC Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu Gln Ile His	702
85 90 95 100	
AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC AGC CGG GCC AAC ACC Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser Arg Ala Asn Thr	750
105 110 115	
GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC CCA GGG ACC Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile Pro Gly Thr	798
120 125 130	
AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC CCT Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile Pro	846
135 140 145	
GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln	894
150 155 160	
GTG GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile	942
165 170 175 180	
TAT GAG GTT ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile	990
185 190 195	
ACA CGA CTA CTG GAC ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn Val Thr Arg Trp	1038
200 205 210	
GAA ACT TTT GAT GTG AGC CCT GCG GTC CTT CGC TGG ACC CGG GAG AAG Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp Thr Arg Glu Lys	1086
215 220 225	
CAG CCA AAC TAT GGG CTA GCC ATT GAG GTG ACT CAC CTC CAT CAG ACT Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His Leu His Gln Thr	1134
230 235 240	
CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC CGA TCG TTA CCT CAA Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg Ser Leu Pro Gln	1182
245 250 255 260	

96

GGG	AGT	GGG	AAT	TGG	GCC	CAG	CTC	CGG	CCC	CTC	CTG	GTC	ACC	TTT	GGC	1230
Gly	Ser	Gly	Asn	Trp	Ala	Gln	Leu	Arg	Pro	Leu	Leu	Val	Thr	Phe	Gly	
			265						270					275		
CAT	GAT	GGC	CGG	GGC	CAT	GCC	TTG	ACC	CGA	CGC	CGG	AGG	GCC	AAG	CGT	1278
His	Asp	Gly	Arg	Gly	His	Ala	Leu	Thr	Arg	Arg	Arg	Arg	Ala	Lys	Arg	
			280					285					290			
AGC	CCT	AAG	CAT	CAC	TCA	CAG	CGG	GCC	AGG	AAG	AAG	AAT	AAG	AAC	TGC	1326
Ser	Pro	Lys	His	His	Ser	Gln	Arg	Ala	Arg	Lys	Lys	Asn	Lys	Asn	Cys	
		295					300					305				
CGG	CGC	CAC	TCG	CTC	TAT	GTG	GAC	TTC	AGC	GAT	GTG	GGC	TGG	AAT	GAC	1374
Arg	Arg	His	Ser	Leu	Tyr	Val	Asp	Phe	Ser	Asp	Val	Gly	Trp	Asn	Asp	
	310					315					320					
TGG	ATT	GTG	GCC	CCA	CCA	GGC	TAC	CAG	GCC	TTC	TAC	TGC	CAT	GGG	GAC	1422
Trp	Ile	Val	Ala	Pro	Pro	Gly	Tyr	Gln	Ala	Phe	Tyr	Cys	His	Gly	Asp	
	325					330				335					340	
TGC	CCC	TTT	CCA	CTG	GCT	GAC	CAC	CTC	AAC	TCA	ACC	AAC	CAT	GCC	ATT	1470
Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser	Thr	Asn	His	Ala	Ile	
				345					350					355		
GTG	CAG	ACC	CTG	GTC	AAT	TCT	GTC	AAT	TCC	AGT	ATC	CCC	AAA	GCC	TGT	1518
Val	Gln	Thr	Leu	Val	Asn	Ser	Val	Asn	Ser	Ser	Ile	Pro	Lys	Ala	Cys	
			360					365					370			
TGT	GTG	CCC	ACT	GAA	CTG	AGT	GCC	ATC	TCC	ATG	CTG	TAC	CTG	GAT	GAG	1566
Cys	Val	Pro	Thr	Glu	Leu	Ser	Ala	Ile	Ser	Met	Leu	Tyr	Leu	Asp	Glu	
		375					380					385				
TAT	GAT	AAG	GTG	GTA	CTG	AAA	AAT	TAT	CAG	GAG	ATG	GTA	GTA	GAG	GGA	1614
Tyr	Asp	Lys	Val	Val	Leu	Lys	Asn	Tyr	Gln	Glu	Met	Val	Val	Glu	Gly	
	390					395				400						
TGT	GGG	TGC	CGC	TGAGATCAGG	CAGTCCTTGA	GGATAGACAG	ATATACACAC									1666
Cys	Gly	Cys	Arg													
	405															
CACACACACA	CACCACATAC	ACCACACACA	CACGTTCCCA	TCCACTCACC	CACACACTAC											1726
ACAGACTGCT	TCCTTATAGC	TGGACTTTTA	TTTAAAAAAA	AAAAAAAAAA	AATGGAAAAA											1786
ATCCCTAAAC	ATTACCTTG	ACCTTATTTA	TGACTTTACG	TGCAAATGTT	TTGACCATAT											1846
TGATCATATA	TTTTGACAAA	ATATATTTAT	AACTACGTAT	TAAAAGAAAA	AAATAAAATG											1906
AGTCATTATT	TTAAAAAAA	AAAAAAA	AACT	CTAGAGTCGA	CGGAATTC											1954

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 408 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ile	Pro	Gly	Asn	Arg	Met	Leu	Met	Val	Val	Leu	Leu	Cys	Gln	Val	1	5	10	15
Leu	Leu	Gly	Gly	Ala	Ser	His	Ala	Ser	Leu	Ile	Pro	Glu	Thr	Gly	Lys	20	25	30	
Lys	Lys	Val	Ala	Glu	Ile	Gln	Gly	His	Ala	Gly	Gly	Arg	Arg	Ser	Gly	35	40	45	
Gln	Ser	His	Glu	Leu	Leu	Arg	Asp	Phe	Glu	Ala	Thr	Leu	Leu	Gln	Met	50	55	60	
Phe	Gly	Leu	Arg	Arg	Arg	Pro	Gln	Pro	Ser	Lys	Ser	Ala	Val	Ile	Pro	65	70	75	80
Asp	Tyr	Met	Arg	Asp	Leu	Tyr	Arg	Leu	Gln	Ser	Gly	Glu	Glu	Glu	Glu	85	90	95	
Glu	Gln	Ile	His	Ser	Thr	Gly	Leu	Glu	Tyr	Pro	Glu	Arg	Pro	Ala	Ser	100	105	110	
Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	His	His	Glu	Glu	His	Leu	Glu	Asn	115	120	125	
Ile	Pro	Gly	Thr	Ser	Glu	Asn	Ser	Ala	Phe	Arg	Phe	Leu	Phe	Asn	Leu	130	135	140	
Ser	Ser	Ile	Pro	Glu	Asn	Glu	Val	Ile	Ser	Ser	Ala	Glu	Leu	Arg	Leu	145	150	155	160
Phe	Arg	Glu	Gln	Val	Asp	Gln	Gly	Pro	Asp	Trp	Glu	Arg	Gly	Phe	His	165	170	175	
Arg	Ile	Asn	Ile	Tyr	Glu	Val	Met	Lys	Pro	Pro	Ala	Glu	Val	Val	Pro	180	185	190	
Gly	His	Leu	Ile	Thr	Arg	Leu	Leu	Asp	Thr	Arg	Leu	Val	His	His	Asn	195	200	205	
Val	Thr	Arg	Trp	Glu	Thr	Phe	Asp	Val	Ser	Pro	Ala	Val	Leu	Arg	Trp	210	215	220	
Thr	Arg	Glu	Lys	Gln	Pro	Asn	Tyr	Gly	Leu	Ala	Ile	Glu	Val	Thr	His	225	230	235	240
Leu	His	Gln	Thr	Arg	Thr	His	Gln	Gly	Gln	His	Val	Arg	Ile	Ser	Arg	245	250	255	
Ser	Leu	Pro	Gln	Gly	Ser	Gly	Asn	Trp	Ala	Gln	Leu	Arg	Pro	Leu	Leu	260	265	270	
Val	Thr	Phe	Gly	His	Asp	Gly	Arg	Gly	His	Ala	Leu	Thr	Arg	Arg	Arg	275	280	285	
Arg	Ala	Lys	Arg	Ser	Pro	Lys	His	His	Ser	Gln	Arg	Ala	Arg	Lys	Lys	290	295	300	

Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val  
 305 310 315 320  
 Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr  
 325 330 335  
 Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 340 345 350  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile  
 355 360 365  
 Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu  
 370 375 380  
 Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met  
 385 390 395 400  
 Val Val Glu Gly Cys Gly Cys Arg  
 405

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1448 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 97..1389

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGACCGAGC GCGCGGACG GCCGCCTGCC CCCTCTGCCA CCTGGGGCGG TGCGGGCCCCG	60
GAGCCCGGAG CCCGGGTAGC GCGTAGAGCC GCGCGC ATG CAC GTG CGC TCA CTG	114
Met His Val Arg Ser Leu	
1 5	
CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA CCC CTG TTC	162
Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro Leu Phe	
10 15 20	
CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC GAG GTG CAC	210
Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu Val His	
25 30 35	
TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG GAG ATG	258
Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg Glu Met	
40 45 50	
CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC CCG CGC CCG	306
Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro Arg Pro	
55 60 65 70	

CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG CTG GAC CTG	354
His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu Asp Leu	
75 80 85	
TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC GGC CAG GGC	402
Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly Gly Gln Gly	
90 95 100	
TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT CTG	450
Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro Leu	
105 110 115	
GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC ATG GTC ATG	498
Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val Met	
120 125 130	
AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC CAC CCA CGC	546
Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro Arg	
135 140 145 150	
TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC CCA GAA GGG	594
Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu Gly	
155 160 165	
GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC TAC ATC CGG	642
Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile Arg	
170 175 180	
GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT CAG GTG CTC	690
Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr Gln Val Leu	
185 190 195	
CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC GAC AGC CGT	738
Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser Arg	
200 205 210	
ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC ATC ACA GCC	786
Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr Ala	
215 220 225 230	
ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG GGC CTG CAG	834
Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu Gln	
235 240 245	
CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG GCG	882
Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu Ala	
250 255 260	
GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC TTC ATG GTG	930
Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met Val	
265 270 275	
GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC CGG TCC ACG	978
Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile Arg Ser Thr	
280 285 290	
GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC AAG AAC CAG	1026
Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn Gln	
295 300 305 310	

100

GAA	GCC	CTG	CGG	ATG	GCC	AAC	GTG	GCA	GAG	AAC	AGC	AGC	AGC	GAC	CAG	1074
Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	Ser	Asp	Gln	
				315					320					325		
AGG	CAG	GCC	TGT	AAG	AAG	CAC	GAG	CTG	TAT	GTC	AGC	TTC	CGA	GAC	CTG	1122
Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	
			330					335					340			
GGC	TGG	CAG	GAC	TGG	ATC	ATC	GCG	CCT	GAA	GGC	TAC	GCC	GCC	TAC	TAC	1170
Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	Ala	Tyr	Tyr	
			345				350					355				
TGT	GAG	GGG	GAG	TGT	GCC	TTC	CCT	CTG	AAC	TCC	TAC	ATG	AAC	GCC	ACC	1218
Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala	Thr	
	360					365					370					
AAC	CAC	GCC	ATC	GTG	CAG	ACG	CTG	GTC	CAC	TTC	ATC	AAC	CCG	GAA	ACG	1266
Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	
	375				380					385					390	
GTG	CCC	AAG	CCC	TGC	TGT	GCG	CCC	ACG	CAG	CTC	AAT	GCC	ATC	TCC	GTC	1314
Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	Leu	Asn	Ala	Ile	Ser	Val	
				395					400					405		
CTC	TAC	TTC	GAT	GAC	AGC	TCC	AAC	GTC	ATC	CTG	AAG	AAA	TAC	AGA	AAC	1362
Leu	Tyr	Phe	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys	Lys	Tyr	Arg	Asn	
			410					415					420			
ATG	GTG	GTC	CGG	GCC	TGT	GGC	TGC	CAC	TAGCTCCTCC	GAGAATTCAG						1409
Met	Val	Val	Arg	Ala	Cys	Gly	Cys	His								
			425				430									
ACCCTTTGGG	GCCAAGTTTT	TCTGGATCCT	CCATTGCTC													1448

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 431 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	His	Val	Arg	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala	
1				5					10					15		
Leu	Trp	Ala	Pro	Leu	Phe	Leu	Leu	Arg	Ser	Ala	Leu	Ala	Asp	Phe	Ser	
			20					25					30			
Leu	Asp	Asn	Glu	Val	His	Ser	Ser	Phe	Ile	His	Arg	Arg	Leu	Arg	Ser	
	35						40					45				
Gln	Glu	Arg	Arg	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	
	50					55					60					
Pro	His	Arg	Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	

65				70				101				75				80			
Met	Phe	Met	Leu	Asp 85	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly				
Gly	Pro	Gly	Gly	100	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser			
Thr	Gln	Gly	Pro	115	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr			
Asp	Ala	Asp	Met	130	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys			
Glu	Phe	Phe	His	145	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu			
Ser	Lys	Ile	Pro	165	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile			
Tyr	Lys	Asp	Tyr	180	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile			
Ser	Val	Tyr	Gln	195	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu			
Phe	Leu	Leu	Asp	210	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu			
Val	Phe	Asp	Ile	225	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg			
His	Asn	Leu	Gly	245	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser			
Ile	Asn	Pro	Lys	260	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn			
Lys	Gln	Pro	Phe	275	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Phe			
Arg	Ser	Ile	Arg	290	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser			
Lys	Thr	Pro	Lys	305	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu			
Asn	Ser	Ser	Ser	325	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr			
Val	Ser	Phe	Arg	340	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu			
Gly	Tyr	Ala	Ala	355	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn			
Ser	Tyr	Met	Asn	370	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His			
Phe	Ile	Asn	Pro		Glu	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln			

102

385		390		395		400
Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile						
	405			410		415
Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His						
	420			425		430

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2923 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: Human placenta

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Stratagene catalog #936203 Human placenta cDNA library
- (B) CLONE: BMP6C35

## (viii) POSITION IN GENOME:

- (C) UNITS: bp

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 160..1701

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 1282..1698

## (ix) FEATURE:

- (A) NAME/KEY: mRNA
- (B) LOCATION: 1..2923

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGACCATGAG AGATAAGGAC TGAGGGCCAG GAAGGGGAAG CGAGCCCGCC GAGAGGTGGC	60
GGGGACTGCT CACGCCAAGG GCCACAGCGG CCGCGCTCCG GCCTCGCTCC GCCGCTCCAC	120
GCCTCGCGGG ATCCGCGGGG GCAGCCCGGC CGGGCGGGG ATG CCG GGG CTG GGG	174
	Met Pro Gly Leu Gly
	-374 -370
CGG AGG GCG CAG TGG CTG TGC TGG TGG TGG GGG CTG CTG TGC AGC TGC	222
Arg Arg Ala Gln Trp Leu Cys Trp Trp Trp Gly Leu Leu Cys Ser Cys	
	-365 -360 -355
TGC GGG CCC CCG CCG CTG CGG CCG CCC TTG CCC GCT GCC GCG GCC GCC	270

Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro Ala Ala Ala Ala Ala	
-350 -345 -340	
GCC GCC GGG GGG CAG CTG CTG GGG GAC GGC GGG AGC CCC GGC CGC ACG	318
Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly Ser Pro Gly Arg Thr	
-335 -330 -325	
GAG CAG CCG CCG CCG TCG CCG CAG TCC TCC TCG GGC TTC CTG TAC CGG	366
Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser Gly Phe Leu Tyr Arg	
-320 -315 -310	
CGG CTC AAG ACG CAG GAG AAG CGG GAG ATG CAG AAG GAG ATC TTG TCG	414
Arg Leu Lys Thr Gln Glu Lys Arg Glu Met Gln Lys Glu Ile Leu Ser	
-305 -300 -295 -290	
GTG CTG GGG CTC CCG CAC CGG CCC CGG CCC CTG CAC GGC CTC CAA CAG	462
Val Leu Gly Leu Pro His Arg Pro Arg Pro Leu His Gly Leu Gln Gln	
-285 -280 -275	
CCG CAG CCC CCG GCG CTC CGG CAG CAG GAG GAG CAG CAG CAG CAG CAG	510
Pro Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu Gln Gln Gln Gln Gln	
-270 -265 -260	
CAG CTG CCT CGC GGA GAG CCC CCT CCC GGG CGA CTG AAG TCC GCG CCC	558
Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg Leu Lys Ser Ala Pro	
-255 -250 -245	
CTC TTC ATG CTG GAT CTG TAC AAC GCC CTG TCC GCC GAC AAC GAC GAG	606
Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser Ala Asp Asn Asp Glu	
-240 -235 -230	
GAC GGG GCG TCG GAG GGG GAG AGG CAG CAG TCC TGG CCC CAC GAA GCA	654
Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser Trp Pro His Glu Ala	
-225 -220 -215 -210	
GCC AGC TCG TCC CAG CGT CGG CAG CCG CCC CCG GGC GCC GCG CAC CCG	702
Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Pro Gly Ala Ala His Pro	
-205 -200 -195	
CTC AAC CGC AAG AGC CTT CTG GCC CCC GGA TCT GGC AGC GGC GGC GCG	750
Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Ser Gly Ser Gly Gly Ala	
-190 -185 -180	
TCC CCA CTG ACC AGC GCG CAG GAC AGC GCC TTC CTC AAC GAC GCG GAC	798
Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe Leu Asn Asp Ala Asp	
-175 -170 -165	
ATG GTC ATG AGC TTT GTG AAC CTG GTG GAG TAC GAC AAG GAG TTC TCC	846
Met Val Met Ser Phe Val Asn Leu Val Glu Tyr Asp Lys Glu Phe Ser	
-160 -155 -150	
CCT CGT CAG CGA CAC CAC AAA GAG TTC AAG TTC AAC TTA TCC CAG ATT	894
Pro Arg Gln Arg His His Lys Glu Phe Lys Phe Asn Leu Ser Gln Ile	
-145 -140 -135 -130	
CCT GAG GGT GAG GTG GTG ACG GCT GCA GAA TTC CGC ATC TAC AAG GAC	942
Pro Glu Gly Glu Val Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp	
-125 -120 -115	
TGT GTT ATG GGG AGT TTT AAA AAC CAA ACT TTT CTT ATC AGC ATT TAT	990

Cys	Val	Met	Gly	Ser	Phe	Lys	Asn	Gln	Thr	Phe	Leu	Ile	Ser	Ile	Tyr	
			-110					-105					-100			
CAA	GTC	TTA	CAG	GAG	CAT	CAG	CAC	AGA	GAC	TCT	GAC	CTG	TTT	TTG	TTG	1038
Gln	Val	Leu	Gln	Glu	His	Gln	His	Arg	Asp	Ser	Asp	Leu	Phe	Leu	Leu	
		-95					-90					-85				
GAC	ACC	CGT	GTA	GTA	TGG	GCC	TCA	GAA	GAA	GGC	TGG	CTG	GAA	TTT	GAC	1086
Asp	Thr	Arg	Val	Val	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Glu	Phe	Asp	
	-80					-75					-70					
ATC	ACG	GCC	ACT	AGC	AAT	CTG	TGG	GTT	GTG	ACT	CCA	CAG	CAT	AAC	ATG	1134
Ile	Thr	Ala	Thr	Ser	Asn	Leu	Trp	Val	Val	Thr	Pro	Gln	His	Asn	Met	
-65					-60					-55					-50	
GGG	CTT	CAG	CTG	AGC	GTG	GTG	ACA	AGG	GAT	GGA	GTC	CAC	GTC	CAC	CCC	1182
Gly	Leu	Gln	Leu	Ser	Val	Val	Thr	Arg	Asp	Gly	Val	His	Val	His	Pro	
			-45						-40					-35		
CGA	GCC	GCA	GGC	CTG	GTG	GGC	AGA	GAC	GGC	CCT	TAC	GAT	AAG	CAG	CCC	1230
Arg	Ala	Ala	Gly	Leu	Val	Gly	Arg	Asp	Gly	Pro	Tyr	Asp	Lys	Gln	Pro	
		-30						-25					-20			
TTC	ATG	GTG	GCT	TTC	TTC	AAA	GTG	AGT	GAG	GTC	CAC	GTG	CGC	ACC	ACC	1278
Phe	Met	Val	Ala	Phe	Phe	Lys	Val	Ser	Glu	Val	His	Val	Arg	Thr	Thr	
	-15					-10					-5					
AGG	TCA	GCC	TCC	AGC	CGG	CGC	CGA	CAA	CAG	AGT	CGT	AAT	CGC	TCT	ACC	1326
Arg	Ser	Ala	Ser	Ser	Arg	Arg	Arg	Gln	Gln	Ser	Arg	Asn	Arg	Ser	Thr	
	1				5					10				15		
CAG	TCC	CAG	GAC	GTG	GCG	CGG	GTC	TCC	AGT	GCT	TCA	GAT	TAC	AAC	AGC	1374
Gln	Ser	Gln	Asp	Val	Ala	Arg	Val	Ser	Ser	Ala	Ser	Asp	Tyr	Asn	Ser	
			20					25						30		
AGT	GAA	TTG	AAA	ACA	GCC	TGC	AGG	AAG	CAT	GAG	CTG	TAT	GTG	AGT	TTC	1422
Ser	Glu	Leu	Lys	Thr	Ala	Cys	Arg	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	
			35				40						45			
CAA	GAC	CTG	GGA	TGG	CAG	GAC	TGG	ATC	ATT	GCA	CCC	AAG	GGC	TAT	GCT	1470
Gln	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Lys	Gly	Tyr	Ala	
	50						55					60				
GCC	AAT	TAC	TGT	GAT	GGA	GAA	TGC	TCC	TTC	CCA	CTC	AAC	GCA	CAC	ATG	1518
Ala	Asn	Tyr	Cys	Asp	Gly	Glu	Cys	Ser	Phe	Pro	Leu	Asn	Ala	His	Met	
	65					70					75					
AAT	GCA	ACC	AAC	CAC	GCG	ATT	GTG	CAG	ACC	TTG	GTT	CAC	CTT	ATG	AAC	1566
Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Leu	Met	Asn	
80					85					90					95	
CCC	GAG	TAT	GTC	CCC	AAA	CCG	TGC	TGT	GCG	CCA	ACT	AAG	CTA	AAT	GCC	1614
Pro	Glu	Tyr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Asn	Ala	
				100					105					110		
ATC	TCG	GTT	CTT	TAC	TTT	GAT	GAC	AAC	TCC	AAT	GTC	ATT	CTG	AAA	AAA	1662
Ile	Ser	Val	Leu	Tyr	Phe	Asp	Asp	Asn	Ser	Asn	Val	Ile	Leu	Lys	Lys	
			115					120					125			
TAC	AGG	AAT	ATG	GTT	GTA	AGA	GCT	TGT	GGA	TGC	CAC	TA	ACTCGAAA			1708

Tyr	Arg	Asn	Met	Val	Val	Arg	Ala	Cys	Gly	Cys	His	
		130					135				140	

CCAGATGCTG	GGGACACACA	TTCTGCCTTG	GATTCCTAGA	TTACATCTGC	CTTAAAAAAA	1768
CACGGAAGCA	CAGTTGGAGG	TGGGACGATG	AGACTTTGAA	ACTATCTCAT	GCCAGTGCCT	1828
TATTACCCAG	GAAGATTTTA	AAGGACCTCA	TTAATAATTT	GCTCACTTGG	TAAATGACGT	1888
GAGTAGTTGT	TGGTCTGTAG	CAAGCTGAGT	TTGGATGTCT	GTAGCATAAG	GTCTGGTAAC	1948
TGCAGAAACA	TAACCGTGAA	GCTCTTCCTA	CCCTCCTCCC	CCAAAAACCC	ACCAAAATTA	2008
GTTTTAGCTG	TAGATCAAGC	TATTTGGGGT	GTTTGTTAGT	AAATAGGGAA	AATAATCTCA	2068
AAGGAGTTAA	ATGTATTCTT	GGCTAAAGGA	TCAGCTGGTT	CAGTACTGTC	TATCAAAGGT	2128
AGATTTTACA	GAGAACAGAA	ATCGGGGAAG	TGGGGGGAAC	GCCTCTGTTC	AGTTCATTCC	2188
CAGAAGTCCA	CAGGACGCAC	AGCCCAGGCC	ACAGCCAGGG	CTCCACGGGG	CGCCCTTGTC	2248
TCAGTCATTG	CTGTTGTATG	TTCGTGCTGG	AGTTTTGTTG	GTGTGAAAAT	ACACTTATTT	2308
CAGCCAAAAC	ATACCATTTC	TACACCTCAA	TCCTCCATTT	GCTGTACTCT	TTGCTAGTAC	2368
CAAAGTAGA	CTGATTACAC	TGAGGTGAGG	CTACAAGGGG	TGTGTAACCG	TGTAACACGT	2428
GAAGGCAGTG	CTCACCTCTT	CTTTACCAGA	ACGGTTCTTT	GACCAGCACA	TTAACTTCTG	2488
GACTGCCGGC	TCTAGTACCT	TTTCAGTAA	GTGGTTCTCT	GCCTTTTTTAC	TATACAGCAT	2548
ACCACGCCAC	AGGGTTAGAA	CCAACGAAGA	AAATAAAATG	AGGGTGCCCA	GCTTATAAGA	2608
ATGGTGTTAG	GGGGATGAGC	ATGCTGTTTA	TGAACGGAAA	TCATGATTTT	CCTGTAGAAA	2668
GTGAGGCTCA	GATTAAATTT	TAGAATATTT	TCTAAATGTC	TTTTTCACAA	TCATGTGACT	2728
GGGAAGGCAA	TTTCATACTA	AACTGATTAA	ATAATACATT	TATAATCTAC	AACTGTTTGC	2788
ACTTACAGCT	TTTTTTGTAA	ATATAAACTA	TAATTTATTG	TCTATTTTAT	ATCTGTTTTG	2848
CTGTGGCGTT	GGGGGGGGGG	CCGGGCTTTT	GGGGGGGGGG	GTTTGTTTGG	GGGGTGTCTG	2908
GGTGTGGGCG	GGCGG					2923

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 513 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Pro	Gly	Leu	Gly	Arg	Arg	Ala	Gln	Trp	Leu	Cys	Trp	Trp	Trp	Gly
-374							-370			-365					-360

106

Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro  
 -355 -350 -345  
 Ala Ala Ala Ala Ala Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly  
 -340 -335 -330  
 Ser Pro Gly Arg Thr Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser  
 -325 -320 -315  
 Gly Phe Leu Tyr Arg Arg Leu Lys Thr Gln Glu Lys Arg Glu Met Gln  
 -310 -305 -300 -295  
 Lys Glu Ile Leu Ser Val Leu Gly Leu Pro His Arg Pro Arg Pro Leu  
 -290 -285 -280  
 His Gly Leu Gln Gln Pro Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu  
 -275 -270 -265  
 Gln Gln Gln Gln Gln Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg  
 -260 -255 -250  
 Leu Lys Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser  
 -245 -240 -235  
 Ala Asp Asn Asp Glu Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser  
 -230 -225 -220 -215  
 Trp Pro His Glu Ala Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Pro  
 -210 -205 -200  
 Gly Ala Ala His Pro Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Ser  
 -195 -190 -185  
 Gly Ser Gly Gly Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe  
 -180 -175 -170  
 Leu Asn Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu Tyr  
 -165 -160 -155  
 Asp Lys Glu Phe Ser Pro Arg Gln Arg His His Lys Glu Phe Lys Phe  
 -150 -145 -140 -135  
 Asn Leu Ser Gln Ile Pro Glu Gly Glu Val Val Thr Ala Ala Glu Phe  
 -130 -125 -120  
 Arg Ile Tyr Lys Asp Cys Val Met Gly Ser Phe Lys Asn Gln Thr Phe  
 -115 -110 -105  
 Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu His Gln His Arg Asp Ser  
 -100 -95 -90  
 Asp Leu Phe Leu Leu Asp Thr Arg Val Val Trp Ala Ser Glu Glu Gly  
 -85 -80 -75  
 Trp Leu Glu Phe Asp Ile Thr Ala Thr Ser Asn Leu Trp Val Val Thr  
 -70 -65 -60 -55  
 Pro Gln His Asn Met Gly Leu Gln Leu Ser Val Val Thr Arg Asp Gly  
 -50 -45 -40

107

Val His Val His Pro Arg Ala Ala Gly Leu Val Gly Arg Asp Gly Pro  
                     -35                    -30                    -25  
 Tyr Asp Lys Gln Pro Phe Met Val Ala Phe Phe Lys Val Ser Glu Val  
                     -20                    -15                    -10  
 His Val Arg Thr Thr Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser  
                     -5                    1                    5                    10  
 Arg Asn Arg Ser Thr Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala  
                     15                    20                    25  
 Ser Asp Tyr Asn Ser Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu  
                     30                    35                    40  
 Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala  
                     45                    50                    55  
 Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe Pro  
                     60                    65                    70  
 Leu Asn Ala His Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu  
                     75                    80                    85                    90  
 Val His Leu Met Asn Pro Glu Tyr Val Pro Lys Pro Cys Cys Ala Pro  
                     95                    100                    105  
 Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Asn Ser Asn  
                     110                    115                    120  
 Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys  
                     125                    130                    135  
 His

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2153 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (H) CELL LINE: U2-OS osteosarcoma

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: U2-OS human osteosarcoma cDNA library
- (B) CLONE: U2-16

## (viii) POSITION IN GENOME:

- (C) UNITS: bp

## (ix) FEATURE:

- (A) NAME/KEY: CDS

(B) LOCATION: 699..2063

(ix) FEATURE:

(A) NAME/KEY: mat\_peptide

(B) LOCATION: 1647..2060

(ix) FEATURE:

(A) NAME/KEY: mRNA

(B) LOCATION: 1..2153

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGTATATT TGTGCCTGCT GGAGGTGGAA TTAACAGTAA GAAGGAGAAA GGGATTGAAT	60
GGACTTACAG GAAGGATTTC AAGTAAATTC AGGGAAACAC ATTTACTTGA ATAGTACAAC	120
CTAGAGTATT ATTTTACACT AAGACGACAC AAAAGATGTT AAAGTTATCA CCAAGCTGCC	180
GGACAGATAT ATATTCCAAC ACCAAGGTGC AGATCAGCAT AGATCTGTGA TTCAGAAATC	240
AGGATTTGTT TTGGAAAGAG CTCAAGGGTT GAGAAGAACT CAAAAGCAAG TGAAGATTAC	300
TTTGGGAACT ACAGTTTATC AGAAGATCAA CTTTGTCTAA TTCAAATACC AAAGGCCTGA	360
TTATCATAAA TTCATATAGG AATGCATAGG TCATCTGATC AAATAATATT AGCCGTCTTC	420
TGCTACATCA ATGCAGCAAA AACTCTTAAC AACTGTGGAT AATTGGAAAT CTGAGTTTCA	480
GCTTCTTAG AAATAACTAC TCTTGACATA TTCCAAAATA TTTAAAATAG GACAGGAAAA	540
TCGGTGAGGA TGTGTGCTC AGAAATGTCA CTGTCATGAA AAATAGGTAA ATTTGTTTTT	600
TCAGCTACTG GGAAACTGTA CCTCCTAGAA CCTTAGGTTT TTTTTTTTTT AAGAGGACAA	660
GAAGGACTAA AAATATCAAC TTTTGCTTTT GGACAAAA ATG CAT CTG ACT GTA	713
Met His Leu Thr Val	
-316-315	
TTT TTA CTT AAG GGT ATT GTG GGT TTC CTC TGG AGC TGC TGG GTT CTA	761
Phe Leu Lys Gly Ile Val Gly Phe Leu Trp Ser Cys Trp Val Leu	
-310 -305 -300	
GTG GGT TAT GCA AAA GGA GGT TTG GGA GAC AAT CAT GTT CAC TCC AGT	809
Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn His Val His Ser Ser	
-295 -290 -285 -280	
TTT ATT TAT AGA AGA CTA CGG AAC CAC GAA AGA CGG GAA ATA CAA AGG	857
Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg Arg Glu Ile Gln Arg	
-275 -270 -265	
GAA ATT CTC TCT ATC TTG GGT TTG CCT CAC AGA CCC AGA CCA TTT TCA	905
Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro Arg Pro Phe Ser	
-260 -255 -250	
CCT GGA AAA ATG ACC AAT CAA GCG TCC TCT GCA CCT CTC TTT ATG CTG	953
Pro Gly Lys Met Thr Asn Gln Ala Ser Ser Ala Pro Leu Phe Met Leu	
-245 -240 -235	
GAT CTC TAC AAT GCC GAA GAA AAT CCT GAA GAG TCG GAG TAC TCA GTA	1001

Asp	Leu	Tyr	Asn	Ala	Glu	Glu	Asn	Pro	Glu	Glu	Ser	Glu	Tyr	Ser	Val	
-230						-225					-220					
AGG	GCA	TCC	TTG	GCA	GAA	GAG	ACC	AGA	GGG	GCA	AGA	AAG	GGA	TAC	CCA	1049
Arg	Ala	Ser	Leu	Ala	Glu	Glu	Thr	Arg	Gly	Ala	Arg	Lys	Gly	Tyr	Pro	
-215					-210				-205						-200	
GCC	TCT	CCC	AAT	GGG	TAT	CCT	CGT	CGC	ATA	CAG	TTA	TCT	CGG	ACG	ACT	1097
Ala	Ser	Pro	Asn	Gly	Tyr	Pro	Arg	Arg	Ile	Gln	Leu	Ser	Arg	Thr	Thr	
				-195					-190					-185		
CCT	CTG	ACC	ACC	CAG	AGT	CCT	CCT	CTA	GCC	AGC	CTC	CAT	GAT	ACC	AAC	1145
Pro	Leu	Thr	Thr	Gln	Ser	Pro	Pro	Leu	Ala	Ser	Leu	His	Asp	Thr	Asn	
			-180					-175					-170			
TTT	CTG	AAT	GAT	GCT	GAC	ATG	GTC	ATG	AGC	TTT	GTC	AAC	TTA	GTT	GAA	1193
Phe	Leu	Asn	Asp	Ala	Asp	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	
	-165					-160						-155				
AGA	GAC	AAG	GAT	TTT	TCT	CAC	CAG	CGA	AGG	CAT	TAC	AAA	GAA	TTT	CGA	1241
Arg	Asp	Lys	Asp	Phe	Ser	His	Gln	Arg	Arg	His	Tyr	Lys	Glu	Phe	Arg	
-150						-145					-140					
TTT	GAT	CTT	ACC	CAA	ATT	CCT	CAT	GGA	GAG	GCA	GTG	ACA	GCA	GCT	GAA	1289
Phe	Asp	Leu	Thr	Gln	Ile	Pro	His	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	
-135				-130						-125					-120	
TTC	CGG	ATA	TAC	AAG	GAC	CGG	AGC	AAC	AAC	CGA	TTT	GAA	AAT	GAA	ACA	1337
Phe	Arg	Ile	Tyr	Lys	Asp	Arg	Ser	Asn	Asn	Arg	Phe	Glu	Asn	Glu	Thr	
				-115					-110					-105		
ATT	AAG	ATT	AGC	ATA	TAT	CAA	ATC	ATC	AAG	GAA	TAC	ACA	AAT	AGG	GAT	1385
Ile	Lys	Ile	Ser	Ile	Tyr	Gln	Ile	Ile	Lys	Glu	Tyr	Thr	Asn	Arg	Asp	
			-100					-95					-90			
GCA	GAT	CTG	TTC	TTG	TTA	GAC	ACA	AGA	AAG	GCC	CAA	GCT	TTA	GAT	GTG	1433
Ala	Asp	Leu	Phe	Leu	Leu	Asp	Thr	Arg	Lys	Ala	Gln	Ala	Leu	Asp	Val	
	-85					-80					-75					
GGT	TGG	CTT	GTC	TTT	GAT	ATC	ACT	GTG	ACC	AGC	AAT	CAT	TGG	GTG	ATT	1481
Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	Val	Thr	Ser	Asn	His	Trp	Val	Ile	
	-70					-65					-60					
AAT	CCC	CAG	AAT	AAT	TTG	GGC	TTA	CAG	CTC	TGT	GCA	GAA	ACA	GGG	GAT	1529
Asn	Pro	Gln	Asn	Asn	Leu	Gly	Leu	Gln	Leu	Cys	Ala	Glu	Thr	Gly	Asp	
-55				-50						-45					-40	
GGA	CGC	AGT	ATC	AAC	GTA	AAA	TCT	GCT	GGT	CTT	GTG	GGA	AGA	CAG	GGA	1577
Gly	Arg	Ser	Ile	Asn	Val	Lys	Ser	Ala	Gly	Leu	Val	Gly	Arg	Gln	Gly	
			-35						-30					-25		
CCT	CAG	TCA	AAA	CAA	CCA	TTC	ATG	GTG	GCC	TTC	TTC	AAG	GCG	AGT	GAG	1625
Pro	Gln	Ser	Lys	Gln	Pro	Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Ser	Glu	
			-20					-15					-10			
GTA	CTT	CTT	CGA	TCC	GTG	AGA	GCA	GCC	AAC	AAA	CGA	AAA	AAT	CAA	AAC	1673
Val	Leu	Leu	Arg	Ser	Val	Arg	Ala	Ala	Asn	Lys	Arg	Lys	Asn	Gln	Asn	
	-5						1				5					
CGC	AAT	AAA	TCC	AGC	TCT	CAT	CAG	GAC	TCC	TCC	AGA	ATG	TCC	AGT	GTT	1721

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 454 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met His Leu Thr Val Phe Leu Leu Lys Gly Ile Val Gly Phe Leu Trp  
-316 -315 -310 -305

Ser Cys Trp Val Leu Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn  
-300 -295 -290 -285

His Val His Ser Ser Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg  
-280 -275 -270

Arg Glu Ile Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg  
-265 -260 -255



```

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
  70                               75                               80
Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
  85                               90                               95
Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
                     105                               110
Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
                     120                               125                               130
Arg Ser Cys Gly Cys His
                     135

```

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1003 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: Human Heart

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Human heart cDNA library stratagene catalog #936208
- (B) CLONE: hH38

## (viii) POSITION IN GENOME:

- (C) UNITS: bp

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 8..850

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 427..843

## (ix) FEATURE:

- (A) NAME/KEY: mRNA
- (B) LOCATION: 1..997

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

GAATTCC GAG CCC CAT TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC
Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile
-139                -135                -130

```

113

CCG GCT GGG GAG GCG GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val -125 -120 -115 -110	9
CCC AGC ATC CAC CTG CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG Pro Ser Ile His Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln -105 -100 -95	14
GTG GTC CAG GAG CAG TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT Val Val Gln Glu Gln Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp -90 -85 -80	19
CTT CAG ACG CTC CGA GCT GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC Leu Gln Thr Leu Arg Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val -75 -70 -65	24
ACA GCA GCC AGT GAC TGC TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA Thr Ala Ala Ser Asp Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly -60 -55 -50	28
CTC CGC CTC TAT GTG GAG ACT GAG GAT GGG CAC AGC GTG GAT CCT GGC Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly -45 -40 -35 -30	33
CTG GCC GGC CTG CTG GGT CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe -25 -20 -15	38
GTG GTC ACT TTC TTC AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG Val Val Thr Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg -10 -5 1	43
GCA GTG AGG CCA CTG AGG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu 5 10 15	48
CCG CAG GCC AAC CGA CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser 20 25 30 35	52
CAC GGC CGG CAG GTC TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln 40 45 50	57
GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala 55 60 65	62
TAT TAC TGT GAG GGG GAG TGC TCC TTC CCG CTG GAC TCC TGC ATG AAC Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn 70 75 80	67
GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro 85 90 95	72
AAC GCA GTC CCC AAG GCG TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr 100 105 110 115	76

114

TCT GTG CTC TAC TAT GAC AGC AGC AAC AAC GTC ATC CTG CGC AAG CAC	817
Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His	
120 125 130	
CGC AAC ATG GTG GTC AAG GCC TGC GGC TGC CAC TGAGTCAGCC CGCCAGCCCC	870
Arg Asn Met Val Val Lys Ala Cys Gly Cys His	
135 140	
TACTGCAGCC ACCCTTCTCA TCTGGATCGG GCCCTGCAGA GGCAGAAAAC CCTTAAATGC	930
TGTCACAGCT CAAGCAGGAG TGTCAGGGGC CCTCACTCTC GGTGCCTACT TCCTGTCAGG	990
CTTCTGGGAA TTC	1003

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 281 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala	-125
-139 -135 -130	
Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser	-110
-120 -115	
Ile His Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val	-95
-105 -100	
Gln Glu Gln Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln	-80
-90 -85	
Thr Leu Arg Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala	-60
-75 -70 -65	
Ala Ser Asp Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg	-45
-55 -50	
Leu Tyr Val Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala	-30
-40 -35	
Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val	-15
-25 -20	
Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val	5
-10 -5 1	
Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln	20
10 15	
Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly	35
25 30 35	
Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu	

115

40						45						50					
Gly	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr		
55						60				65							
Cys	Glu	Gly	Glu	Cys	Ser	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	Thr		
70				75						80				85			
Asn	His	Ala	Ile	Leu	Gln	Ser	Leu	Val	His	Leu	Met	Lys	Pro	Asn	Ala		
				90				95						100			
Val	Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr	Ser	Val		
		105						110				115					
Leu	Tyr	Tyr	Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg	Lys	His	Arg	Asn		
		120				125						130					
Met	Val	Val	Lys	Ala	Cys	Gly	Cys	His									
135						140											

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2623 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: pALBP2-781

(ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 2724..3071

(ix) FEATURE:

- ```
(A) NAME/KEY: terminator
(B) LOCATION: 3150..3218
```

(ix) FEATURE:

- (A) NAME/KEY: RBS  
(B) LOCATION: 2222..2723

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| GACGAAAGGG | CCTCGTGATA | CGCCTATTTT | TATAGGTAA  | TGTCATGATA | ATAATGGTTT | 60  |
| CTTAGACGTC | AGGTGGCACT | TTTCGGGGAA | ATGTGCGCGG | AACCCCTATT | TGTTTATTTT | 120 |
| TCTAAATACA | TTCAAATATG | TATCCGCTCA | TGAGACAATA | ACCCTGATAA | ATGCTTCAAT | 180 |
| AATATTGAAA | AAGGAAGAGT | ATGAGTATTC | AACATTTCCG | TGTCGCCCTT | ATTCCCTTTT | 240 |
| TTGCGGCATT | TTGCCTTCCT | GTTTTTGCTC | ACCCAGAAAC | GCTGGTGAAA | GTAAAAGATG | 300 |
| CTGAAGATCA | GTTGGGTGCA | CGAGTGGGTT | ACATCGAACT | GGATCTCAAC | AGCGGTAAGA | 360 |

116

|             |            |             |            |            |            |      |
|-------------|------------|-------------|------------|------------|------------|------|
| TCCTTGAGAG  | TTTTCGCCCC | GAAGAACGTT  | TTCCAATGAT | GAGCACTTTT | AAAGTTCTGC | 420  |
| TATGTGGCGC  | GGTATTATCC | CGTATTGACG  | CCGGGCAAGA | GCAACTCGGT | CGCCGCATAC | 480  |
| ACTATTCTCA  | GAATGACTTG | GTTGAGTACT  | CACCAGTCAC | AGAAAAGCAT | CTTACGGATG | 540  |
| GCATGACAGT  | AAGAGAATTA | TGCAGTGCTG  | CCATAACCAT | GAGTGATAAC | ACTGCGGCCA | 600  |
| ACTTACTTCT  | GACAACGATC | GGAGGACCGA  | AGGAGCTAAC | CGCTTTTTTG | CACAACATGG | 660  |
| GGGATCATGT  | AACTCGCCTT | GATCGTTGGG  | AACCGGAGCT | GAATGAAGCC | ATACCAAACG | 720  |
| ACGAGCGTGA  | CACCACGATG | CCTGTAGCAA  | TGGCAACAAC | GTTGCGCAA  | CTATTAACTG | 780  |
| GCGAACTACT  | TACTCTAGCT | TCCCGGCAAC  | AATTAATAGA | CTGGATGGAG | GCGGATAAAG | 840  |
| TTGCAGGACC  | ACTTCTGCGC | TCGGCCCTTC  | CGGCTGGCTG | GTTTATTGCT | GATAAATCTG | 900  |
| GAGCCGGTGA  | GCGTGGGTCT | CGCGGTATCA  | TTGCAGCACT | GGGGCCAGAT | GGTAAGCCCT | 960  |
| CCCGTATCGT  | AGTTATCTAC | ACGACGGGGA  | GTCAGGCAAC | TATGGATGAA | CGAAATAGAC | 1020 |
| AGATCGCTGA  | GATAGGTGCC | TCACTGATTA  | AGCATTGGTA | ACTGTCAGAC | CAAGTTTACT | 1080 |
| CATATATACT  | TTAGATTGAT | TTAAAAC TTC | ATTTTTAATT | TAAAAGGATC | TAGGTGAAGA | 1140 |
| TCCTTTTTGA  | TAATCTCATG | ACCAAAATCC  | CTTAACGTGA | GTTTTTCGTC | CACTGAGCGT | 1200 |
| CAGACCCCGT  | AGAAAAGATC | AAAGGATCTT  | CTTGAGATCC | TTTTTTTCTG | CGCGTAATCT | 1260 |
| GCTGCTTGCA  | AACAAAAAAA | CCACCGCTAC  | CAGCGGTGGT | TTGTTTGCCG | GATCAAGAGC | 1320 |
| TACCAACTCT  | TTTTCCGAAG | GTAAGTGGCT  | TCAGCAGAGC | GCAGATACCA | AATACTGTCC | 1380 |
| TTCTAGTGTA  | GCCGTAGTTA | GGCCACCACT  | TCAAGAACTC | TGTAGCACCG | CCTACATACC | 1440 |
| TCGCTCTGCT  | AATCCTGTTA | CCAGTGGCTG  | CTGCCAGTGG | CGATAAGTCG | TGTCTTACCG | 1500 |
| GGTTGGACTC  | AAGACGATAG | TTACCGGATA  | AGGCGCAGCG | GTCGGGCTGA | ACGGGGGGTT | 1560 |
| CGTGACACACA | GCCCAGCTTG | GAGCGAACGA  | CCTACACCGA | ACTGAGATAC | CTACAGCGTG | 1620 |
| AGCATTGAGA  | AAGCGCCACG | CTTCCCGAAG  | GGAGAAAGGC | GGACAGGTAT | CCGGTAAGCG | 1680 |
| GCAGGGTCGG  | AACAGGAGAG | CGCACGAGGG  | AGCTTCCAGG | GGGAAACGCC | TGGTATCTTT | 1740 |
| ATAGTCCTGT  | CGGGTTTCGC | CACCTCTGAC  | TTGAGCGTCG | ATTTTTGTGA | TGCTCGTCAG | 1800 |
| GGGGGCGGAG  | CCTATGGAAA | AACGCCAGCA  | ACGCGGCCTT | TTTACGGTTC | CTGGCCTTTT | 1860 |
| GCTGGCCTTT  | TGCTCACATG | TTCTTTCCTG  | CGTTATCCCC | TGATTCTGTG | GATAACCGTA | 1920 |
| TTACCGCCTT  | TGAGTGAGCT | GATACCGCTC  | GCCGCAGCCG | AACGACCGAG | CGCAGCGAGT | 1980 |
| CAGTGAGCGA  | GGAAGCGGAA | GAGCGCCCAA  | TACGCAAACC | GCCTCTCCCC | GCGCGTTGGC | 2040 |
| CGATTCATTA  | ATGCAGAATT | GATCTCTCAC  | CTACCAAACA | ATGCCCCCCT | GCAAAAAATA | 2100 |
| AATTCATATA  | AAAAACATAC | AGATAACCAT  | CTGCGGTGAT | AAATTATCTC | TGGCGGTGTT | 2160 |

117

|             |             |             |                |             |            |      |
|-------------|-------------|-------------|----------------|-------------|------------|------|
| GACATAAATA  | CCACTGGCGG  | TGATACTGAG  | CACATCAGCA     | GGACGCACTG  | ACCACCATGA | 2220 |
| AGGTGACGCT  | CTTAAAAATT  | AAGCCCTGAA  | GAAGGGCAGC     | ATTCAAAGCA  | GAAGGCTTTG | 2280 |
| GGGTGTGTGA  | TACGAAACGA  | AGCATTGGCC  | GTAAGTGCGA     | TTCCGGATTA  | GCTGCCAATG | 2340 |
| TGCCAATCGC  | GGGGGGTTTT  | CGTTCAGGAC  | TACAAC TGCC    | ACACACCACC  | AAAGCTAACT | 2400 |
| GACAGGAGAA  | TCCAGATGGA  | TGCACAAACA  | CGCCGCCGCG     | AACGTCGCGC  | AGAGAAACAG | 2460 |
| GCTCAATGGA  | AAGCAGCAAA  | TCCCCTGTTG  | GTTGGGGTAA     | GCGCAAAACC  | AGTTCCGAAA | 2520 |
| GATTTTTTTTA | ACTATAAACG  | CTGATGGAAG  | CGTTTATGCG     | GAAGAGGTAA  | AGCCCTTCCC | 2580 |
| GAGTAACAAA  | AAAACAACAG  | CATAAATAAC  | CCCGCTCTTA     | CACATTCCAG  | CCCTGAAAAA | 2640 |
| GGGCATCAAA  | TTAAACCACA  | CCTATGGTGT  | ATGCATTTAT     | TTGCATACAT  | TCAATCAATT | 2700 |
| GTATCTAAG   | GAAATACTTA  | CAT ATG CAA | GCT AAA CAT    | AAA CAA CGT | AAA        | 2750 |
|             |             | Met Gln Ala | Lys His Lys    | Gln Arg Lys |            |      |
|             |             | 1           |                | 5           |            |      |
| CGT CTG AAA | TCT AGC TGT | AAG AGA CAC | CCT TTG TAC    | GTG GAC TTC | AGT        | 2798 |
| Arg Leu Lys | Ser Ser Cys | Lys Lys Arg | His Pro Leu    | Tyr Val Asp | Phe Ser    |      |
| 10          |             | 15          |                | 20          | 25         |      |
| GAC GTG GGG | TGG AAT GAC | TGG ATT GTG | GCT CCC CCG    | GGG TAT CAC | GCC        | 2846 |
| Asp Val Gly | Trp Asn Asp | Trp Ile Val | Ala Pro Pro    | Gly Tyr His | Ala        |      |
|             | 30          |             | 35             |             | 40         |      |
| TTT TAC TGC | CAC GGA GAA | TGC CCT TTT | CCT CTG GCT    | GAT CAT CTG | AAC        | 2894 |
| Phe Tyr Cys | His Gly Glu | Cys Pro Phe | Pro Leu Ala    | Asp His Leu | Asn        |      |
|             | 45          |             | 50             |             | 55         |      |
| TCC ACT AAT | CAT GCC ATT | GTT CAG ACG | TTG GTC AAC    | TCT GTT AAC | TCT        | 2942 |
| Ser Thr Asn | His Ala Ile | Val Gln Thr | Leu Val Asn    | Ser Val Asn | Ser        |      |
|             | 60          |             | 65             |             | 70         |      |
| AAG ATT CCT | AAG GCA TGC | TGT GTC CCG | ACA GAA CTC    | AGT GCT ATC | TCG        | 2990 |
| Lys Ile Pro | Lys Ala Cys | Cys Val Pro | Thr Glu Leu    | Ser Ala Ile | Ser        |      |
|             | 75          |             | 80             |             | 85         |      |
| ATG CTG TAC | CTT GAC GAG | AAT GAA AAG | GTT GTA TTA    | AAG AAC TAT | CAG        | 3038 |
| Met Leu Tyr | Leu Asp Glu | Asn Glu Lys | Val Val Leu    | Lys Asn Tyr | Gln        |      |
|             | 90          |             | 95             |             | 100        | 105  |
| GAC ATG GTT | GTG GAG GGT | TGT GGG TGT | CGC TAGTACAGCA | AAATTAAATA  |            | 3088 |
| Asp Met Val | Val Glu Gly | Cys Gly Cys | Arg            |             |            |      |
|             | 110         |             | 115            |             |            |      |
| CATAAATATA  | TATATATATA  | TATATTTTAG  | AAAAAAGAAA     | AAAATCTAGA  | GTCGACCTGC | 3148 |
| AGTAATCGTA  | CAGGGTAGTA  | CAAATAAAAA  | AGGCACGTCA     | GATGACGTGC  | CTTTTTTCTT | 3208 |
| GTGAGCAGTA  | AGCTTGGCAC  | TGGCCGTCGT  | TTTACAACGT     | CGTGACTGGG  | AAAACCCTGG | 3268 |
| CGTTACCCAA  | CTTAATCGCC  | TTGCAGCACA  | TCCCCCTTTC     | GCCAGCTGGC  | GTAATAGCGA | 3328 |
| AGAGGGCCCGC | ACCGATCGCC  | CTTCCCAACA  | GTTGCGCAGC     | CTGAATGGCG  | AATGGCGCCT | 3388 |

## 118

|                                                                   |      |
|-------------------------------------------------------------------|------|
| GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAT ATGGTGCACT | 3448 |
| CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGC CCCGACACCC GCCAACACCC | 3508 |
| GCTGACGCGC CCTGACGGGC TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC | 3568 |
| GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGA      | 3623 |

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 115 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Gln | Ala | Lys | His | Lys | Gln | Arg | Lys | Arg | Leu | Lys | Ser | Ser | Cys | Lys |     |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
| Arg | His | Pro | Leu | Tyr | Val | Asp | Phe | Ser | Asp | Val | Gly | Trp | Asn | Asp | Trp |     |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| Ile | Val | Ala | Pro | Pro | Gly | Tyr | His | Ala | Phe | Tyr | Cys | His | Gly | Glu | Cys |     |
|     |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| Pro | Phe | Pro | Leu | Ala | Asp | His | Leu | Asn | Ser | Thr | Asn | His | Ala | Ile | Val |     |
|     |     |     | 50  |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |
| Gln | Thr | Leu | Val | Asn | Ser | Val | Asn | Ser | Lys | Ile | Pro | Lys | Ala | Cys | Cys |     |
|     |     |     | 65  |     |     | 70  |     |     |     | 75  |     |     |     | 80  |     |     |
| Val | Pro | Thr | Glu | Leu | Ser | Ala | Ile | Ser | Met | Leu | Tyr | Leu | Asp | Glu | Asn |     |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
| Glu | Lys | Val | Val | Leu | Lys | Asn | Tyr | Gln | Asp | Met | Val | Val | Glu | Gly | Cys |     |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| Gly | Cys | Arg |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 115 |

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CATGGGCAGC TGAG

14

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 41 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAGGGTTGTG GGTGTCGCTA GTGAGTCGAC TACAGCAAAT T

41

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGATGTGGGT GCCGCTGACT CTAGAGTCGA CGGAATTC

38

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AATTCACCAT GATTCCTGGT AACCGAATGC T

31

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTGGTACTAA GGACCATTGG CTTAC

25

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGACCTGCAG CCATGCATCT GACTGTA

27

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGCCTGCAGT TTAATATTAG TGGCAGC

27

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGACCTGCAG CCACC

15

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 81 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCGACCCACC ATGCCGGGGC TGGGGCGGAG GCGCAGTGG CTGTGCTGGT GGTGGGGGCT 60  
GTGCTGCAGC TGCTGCGGGC C 81

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGCAGCAGCT GCACAGCAGC CCCCACCACC AGCACAGCCA CTGCGCCCTC CGCCCCAGCC 60  
CCGGCATGGT GGG 73

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCGACTGGTT T 11

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGAAACCAG

122

9

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCGACAGGCT CGCCTGCA

18

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 10 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTCCGAGCGG

10

## (2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CAGGTCGACC CACCATGCAC GTGCGCTCA

29

## (2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

123

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCTGTCGACC TCGGAGGAGC TAGTGGC

27

## WHAT IS CLAIMED IS:

1. A method for producing a heterodimeric protein having bone stimulating activity comprising culturing a selected host cell containing a sequence encoding a first selected BMP or fragment thereof and a sequence encoding a second selected BMP or fragment thereof, said sequences each being under the control of a suitable regulatory sequence capable of directing co-expression of said proteins, and isolating said heterodimeric protein from the culture medium.

2. The method according to claim 1 wherein said first BMP or fragment thereof is present on a first vector transfected into said host cell and said second BMP or fragment thereof is present on a second vector transfected into said host cell.

3. The method according to claim 1 wherein both said BMPs or fragments thereof are incorporated into a chromosome of said host cell.

4. The method according to claim 1 wherein both BMPs or fragments thereof are present on a single vector.

5. The method according to claim 2 wherein

more than a single copy of the gene encoding each said BMP or fragment thereof is present on each vector.

6. The method according to claim 1 wherein said host cell is a hybrid cell prepared by culturing two fused selected, stable host cells, each host cell transfected with a sequence encoding a selected first or second BMP or fragment thereof, said sequences under the control of a suitable regulatory sequence capable of directing expression of each protein or fragment.

7. The method according to claim 1 wherein said host cell is a mammalian cell.

8. The method according to claim 1 wherein said host cell is an insect cell.

9. The method according to claim 1 wherein said host cell is a yeast cell.

10. A method for producing a heterodimeric protein having bone stimulating activity in a bacterial cell comprising culturing a selected host cell containing a sequence encoding a first selected BMP or fragment thereof under the control of a suitable regulatory sequence capable of directing expression of the protein or protein fragment under conditions suitable for the

126

formation of a soluble, monomeric protein; culturing a  
selected host cell containing a sequence encoding a  
second selected BMP or fragment thereof under the control  
of a suitable regulatory sequence capable of directing  
5 expression of the protein or protein fragment under said  
conditions to form a second soluble, monomeric protein;  
and mixing said soluble monomeric proteins under  
conditions permitting the formation of dimeric proteins  
associated by at least one covalent disulfide bond;  
10 isolating from the mixture a heterodimeric protein.

11. The method according to claim 10 wherein  
said host cell is *E. coli*.

12. The method according to claim 10 wherein  
said conditions comprise treating said protein with a  
15 solubilizing agent.

13. A recombinant heterodimeric protein having  
bone stimulating activity comprising a first protein or  
fragment of BMP-2 in association with a second protein or  
fragment thereof selected from the group consisting of  
20 BMP-5, BMP-6, BMP-7 and BMP-8.

14. The protein according to claim 13 wherein  
said second protein is BMP-5.

127

15. The protein according to claim 13 wherein  
said second protein is BMP-6.

16. The protein according to claim 13 wherein  
said second protein is BMP-7.

5 17. The protein according to claim 13 wherein  
said second protein is BMP-8.

18. A recombinant heterodimeric protein having  
bone stimulating activity comprising a protein or  
fragment of BMP-4 in association with a second protein or  
10 fragment thereof selected from the group consisting of  
BMP-5, BMP-6, BMP-7 and BMP-8.

19. The protein according to claim 18 wherein  
said second protein is BMP-5.

20. The protein according to claim 18 wherein  
15 said second protein is BMP-6.

21. The protein according to claim 18 wherein  
said second protein is BMP-7.

22. The protein according to claim 18 wherein  
said second protein is BMP-8.

23. A recombinant heterodimeric protein having bone stimulating activity comprising a protein or fragment of a first BMP in association with a second protein or fragment of a second BMP produced by co-expressing said proteins in a selected host cell.

24. The protein according to claim 23 wherein said first BMP is BMP-2 and said second BMP is BMP-7.

25. A cell line comprising a nucleotide sequence encoding a first BMP or fragment thereof under control of a suitable expression regulatory system and a nucleotide sequence encoding a second BMP or fragment thereof under control of a suitable expression regulatory system, said regulatory systems capable of directing the co-expression of said BMPs or fragments thereof and the formation of heterodimeric protein.

26. The cell line according to claim 25 wherein said nucleotide sequences encoding said first and second BMP proteins are present in a single DNA molecule.

27. The cell line according to claim 25 wherein said nucleotide sequence encoding said first BMP is present on a first DNA molecule and said nucleotide sequence encoding said second BMP is present on a second DNA molecule.

28. The cell line according to claim 26 wherein said single DNA molecule comprises a first transcription unit containing a gene encoding a first BMP or fragment thereof and a second transcription unit  
5 containing a gene encoding a second BMP or fragment thereof.

29. The cell line according to claim 26 wherein said single DNA molecule comprises a single transcription unit containing multiple copies of said  
10 gene encoding said first BMP or fragments thereof and multiple copies of said gene encoding said second BMP or fragments thereof.

30. A DNA molecule comprising a sequence encoding a first selected BMP or fragment thereof and a  
15 sequence encoding a second selected BMP or fragment thereof, said sequences under the control of at least one suitable regulatory sequence capable of directing co-expression of each BMP or fragment thereof.

31. The molecule according to claim 30  
20 comprising a first transcription unit containing a gene encoding a first BMP or fragment thereof and a second transcription unit containing a gene encoding a second BMP or fragment thereof.

32. The molecule according to claim 30 comprising a single transcription unit containing multiple copies of said gene encoding said first BMP or fragments thereof and multiple copies of said gene  
5 encoding said second BMP or fragments thereof.

33. The protein according to claim 23 wherein said first BMP is BMP-2 and said second BMP is BMP-6.

34. A recombinant BMP-2 homodimer having bone stimulating activity said homodimer produced in E. coli.

10 35. A method for producing a homodimeric BMP-2 protein having bone stimulating activity said method comprising culturing E. coli host cells and isolating and purifying said protein from the resulting culture medium.

15 36. A recombinant heterodimeric protein having bone stimulating activity comprising a first protein or fragment of BMP-2 in association with a second protein or fragment of BMP-2.

## FIGURE 1A

10 20 30 40 50 60 70  
 GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT AACTTGCGCA  
 80 90 100 110 120 130 140  
 CCCCACCTTG CGCCGGTGCC TTTGCCCCAG CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCCTCC  
 150 160 170 180 190 200 210  
 ACTCCTCGGC CTTGCCCCGAC ACTGAGACGC TGTTCACAGC GTGAAAAGAG AGACTGCGCG GCCGGCACCC  
 220 230 240 250 260 270 280  
 GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTCGGT CCTTGCGCCA GGTCTTTTGA CCAGAGTTTT  
 290 300 310 320 330 340 350  
 TCCATGTGGA CGCTCTTTCA ATGGACGTGT CCCC GCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT  
 (1) 370 385 400  
 CGACC ATG GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 415 430 445  
 CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 (24)  
 460 475 490 505  
 GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 520 535 550 565  
 TTC GAG TTG CGG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 580 595 610  
 AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCA GGT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 625 640 655 670  
 CAG CCG GGC TCA CCC GCC CCA GAC CAC CGG TTG GAG AGG GCA GCC AGC CGA GCC  
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala

SUBSTITUTE SHEET

## FIGURE 1B

685 700 715  
 AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG  
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr  
 730 745 760 775  
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
 850 865 880  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
 895 910 925 940  
 CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGA CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu Asp Thr Arg Leu  
 955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
 1000 1015 1030 1045  
 CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
 1060 1075 1090 1105  
 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 (249)  
 1120 1135 1150  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 (266)  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 (283)  
 1225 1240 1255  
 AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 (296)  
 1270 1285 1300 1315  
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala

SUBSTITUTE SHEET

## FIGURE 1C

```

      1330      1345      1360      1375
TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT
Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
      1390      1405      1420
AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG
Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

      1435      1450      1465      1480
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu

      1495      1510      1525
AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

1540(396)      1553      1563      1573      1583      1593      1603
TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
Cys Arg

```

AAAA

## FIGURE 2A

10 20 30 40 50 60 70  
 CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCAGC CCGGAAGCTA GGTGAGTGTG  
 80 90 100 110 120 130 140  
 GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC  
 150 160 170 180 190 200 210  
 GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC AGGTTCAC TG  
 220 230 240 250 260 270 280  
 CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC  
 290 300 310 320 330 340 350  
 GTAGTGCCAT CCGGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG  
 360 370 380 390 400 (1)  
 CTGTCAAGAA TCATGGACTG TTATTATATG CTTGTTTTTC TGTCAAGACA CC ATG ATT CCT  
 MET Ile Pro  
 417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 477 492 507  
 AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln  
 522 537 552 567  
 GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 582 597 612 627  
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 642 657 672  
 AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

SUBSTITUTE SHEET

# SUBSTITUTE SHEET

## FIGURE 2C

1332 1347 1362 1377  
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

1392 1407 1422 1437  
 GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG  
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

1452 1467 1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497 1512 1527 1542  
 GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC  
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile

1557 1572 1587  
 TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu

1602 1617 (408) 1636 1646 1656  
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG  
MET Val Val Glu Gly Cys Gly Cys Arg

1666 1676 1686 1696 1706 1716 1726  
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC

1736 1746 1756 1766 1776 1786 1796  
 ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC

1806 1816 1826 1836 1846 1856 1866  
 ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA

1876 1886 1896 1906 1916 1926 1936  
 ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAATG AGTCATTATT TAAAAAAAAA AAAAAAACT

1946  
 CTAGAGTCGA CGGAATTC

SUBSTITUTE SHEET

## FIGURE 3A

```

      10      20      30      40      50
GTGACCGAGC GCGCGGACG GCCGCCTGCC CCCTCTGCCA CCTGGGGCGG

      60      70      80      90      99
TGCGGGCCCG GAGCCCGGAG CCCGGGTAGC GCGTAGAGCC GGCGCG ATG
  MET
  (1)

      108      117      126      135      144
CAC GTG CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG
His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala

      153      162      171      180      189
CTC TGG GCA CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe

      198      207      216      225      234
AGC CTG GAC AAC GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC
Ser Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu

      243      252      261      270      279
CGC AGC CAG GAG CGG CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT
Arg Ser Gln Glu Arg Arg Glu MET Gln Arg Glu Ile Leu Ser Ile

      288      297      306      315      324
TTG GGC TTG CCC CAC CGC CCG CGC CCG CAC CTC CAG GGC AAG CAC
Leu Gly Leu Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His

      333      342      351      360      369
AAC TCG GCA CCC ATG TTC ATG CTG GAC CTG TAC AAC GCC ATG GCG
Asn Ser Ala Pro MET Phe MET Leu Asp Leu Tyr Asn Ala MET Ala

      378      387      396      405      414
GTG GAG GAG GGC GGC GGG CCC GGC GGC CAG GGC TTC TCC TAC CCC
Val Glu Glu Gly Gly Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro

      423      432      441      450      459
TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT CTG GCC AGC CTG
Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro Leu Ala Ser Leu

      468      477      486      495      504
CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC ATG GTC ATG AGC TTC
Gln Asp Ser His Phe Leu Thr Asp Ala Asp MET Val MET Ser Phe

      513      522      531      540      549
GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC CAC CCA CGC TAC
Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro Arg Tyr

```

SUBSTITUTE SHEET

## FIGURE 3B

|                                                                    |      |      |      |      |
|--------------------------------------------------------------------|------|------|------|------|
| 558                                                                | 567  | 576  | 585  | 594  |
| CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC CCA GAA GGG        |      |      |      |      |
| His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu Gly        |      |      |      |      |
| 603                                                                | 612  | 621  | 630  | 639  |
| GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC TAC ATC        |      |      |      |      |
| Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile        |      |      |      |      |
| 648                                                                | 657  | 666  | 675  | 684  |
| CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT CAG        |      |      |      |      |
| Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr Gln        |      |      |      |      |
| 693                                                                | 702  | 711  | 720  | 729  |
| GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC        |      |      |      |      |
| Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu        |      |      |      |      |
| 738                                                                | 747  | 756  | 765  | 774  |
| GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT        |      |      |      |      |
| Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe        |      |      |      |      |
| 783                                                                | 792  | 801  | 810  | 819  |
| GAC ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC        |      |      |      |      |
| Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His        |      |      |      |      |
| 828                                                                | 837  | 846  | 855  | 864  |
| AAC CTG GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC        |      |      |      |      |
| Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser        |      |      |      |      |
| 873                                                                | 882  | 891  | 900  | 909  |
| ATC AAC CCC AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG        |      |      |      |      |
| Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln        |      |      |      |      |
| 918                                                                | 927  | 936  | 945  | 954  |
| AAC AAG CAG CCC TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC        |      |      |      |      |
| Asn Lys Gln Pro Phe MET Val Ala Phe Phe Lys Ala Thr Glu Val        |      |      |      |      |
| 963                                                                | 972  | 981  | 990  | 999  |
| CAC TTC CGC AGC ATC CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG        |      |      |      |      |
| His Phe Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln        |      |      |      |      |
| (293)                                                              |      |      |      |      |
| 1008                                                               | 1017 | 1026 | 1035 | 1044 |
| AAC CGC TCC AAG ACG CCC AAG AAC CAG GAA GCC CTG CGG ATG GCC        |      |      |      |      |
| Asn Arg Ser Lys Thr Pro Lys <u>Asn Gln Glu Ala Leu Arg</u> MET Ala |      |      |      |      |
| 1053                                                               | 1062 | 1071 | 1080 | 1089 |
| AAC GTG GCA GAG AAC AGC AGC AGC GAC CAG AGG CAG GCC TGT AAG        |      |      |      |      |
| Asn Val Ala Glu Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys        |      |      |      |      |

SUBSTITUTE SHEET

9/32

## FIGURE 3C

1098 1107 1116 1125 1134  
 AAG CAC GAG CTG TAT GTC AGC TTC CGA GAC CTG GGC TGG CAG GAC  
 Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln Asp

1143 1152 1161 1170 1179  
 TGG ATC ATC GCG CCT GAA GGC TAC GCC GCC TAC TAC TGT GAG GGG  
 Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly

1188 1197 1206 1215 1224  
 GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC ACC AAC CAC  
 Glu Cys Ala Phe Pro Leu Asn Ser Tyr MET Asn Ala Thr Asn His

1233 1242 1251 1260 1269  
 GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC CCG GAA ACG GTG  
 Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Ile Ser Val

1278 1287 1296 1305 1314  
 CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC ATC TCC GTC  
 Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val

1323 1332 1341 1350 1359  
 CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA TAC AGA  
 Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg

1368 1377 1386 1399  
 AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC  
 Asn MET Val Val Arg Ala Cys Gly Cys His  
 (431)

1409 1419 1429 1439 1448  
 GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTC

SUBSTITUTE SHEET

10/32

## FIGURE 4A

10 20 30 40 50  
 CGACCATGAG AGATAAGGAC TGAGGGCCAG GAAGGGGAAG CGAGCCCGCC  
 60 70 80 90 100  
 GAGAGGTGGC GGGGACTGCT CACGCCAAGG GCCACAGCGG CCGCGCTCCG  
 110 120 130 140 150  
 GCCTCGCTCC GCCGCTCCAC GCCTCGCGGG ATCCGCGGGG GCAGCCCGGC  
 159 168 177 186 195  
 CGGGCGGGG ATG CCG GGG CTG GGG CGG AGG GCG CAG TGG CTG TGC  
 MET Pro Gly Leu Gly Arg Arg Ala Gln Trp Leu Cys  
 (1)  
 204 213 222 231 240  
 TGG TGG TGG GGG CTG CTG TGC AGC TGC TGC GGG CCC CCG CCG CTG  
 Trp Trp Trp Gly Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu  
 249 258 267 276 285  
 CGG CCG CCC TTG CCC GCT GCC GCG GCC GCC GCC GCG GGG GGG CAG  
 Arg Pro Pro Leu Pro Ala Ala Ala Ala Ala Ala Ala Gly Gly Gln  
 294 303 312 321 330  
 CTG CTG GGG GAC GGC GGG AGC CCC GGC CGC ACG GAG CAG CCG CCG  
 Leu Leu Gly Asp Gly Gly Ser Pro Gly Arg Thr Glu Gln Pro Pro  
 339 348 357 366 375  
 CCG TCG CCG CAG TCC TCC TCG GGC TTC CTG TAC CGG CGG CTC AAG  
 Pro Ser Pro Gln Ser Ser Ser Gly Phe Leu Tyr Arg Arg Leu Lys  
 384 393 402 411 420  
 ACG CAG GAG AAG CGG GAG ATG CAG AAG GAG ATC TTG TCG GTG CTG  
 Thr Gln Glu Lys Arg Glu MET Gln Lys Glu Ile Leu Ser Val Leu  
 429 438 447 456 465  
 GGG CTC CCG CAC CGG CCC CGG CCC CTG CAC GGC CTC CAA CAG CCG  
 Gly Leu Pro His Arg Pro Arg Pro Leu His Gly Leu Gln Gln Pro

SUBSTITUTE SHEET

## FIGURE 4B

|                                                             |     |     |     |     |
|-------------------------------------------------------------|-----|-----|-----|-----|
| 474                                                         | 483 | 492 | 501 | 510 |
| CAG CCC CCG GCG CTC CGG CAG CAG GAG GAG CAG CAG CAG CAG CAG |     |     |     |     |
| Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu Gln Gln Gln Gln Gln |     |     |     |     |
| 519                                                         | 528 | 537 | 546 | 555 |
| CAG CTG CCT CGC GGA GAG CCC CCT CCC GGG CGA CTG AAG TCC GCG |     |     |     |     |
| Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg Leu Lys Ser Ala |     |     |     |     |
| 564                                                         | 573 | 582 | 591 | 600 |
| CCC CTC TTC ATG CTG GAT CTG TAC AAC GCC CTG TCC GCC GAC AAC |     |     |     |     |
| Pro Leu Phe MET Leu Asp Leu Tyr Asn Ala Leu Ser Ala Asp Asn |     |     |     |     |
| 609                                                         | 618 | 627 | 636 | 645 |
| GAC GAG GAC GGG GCG TCG GAG GGG GAG AGG CAG CAG TCC TGG CCC |     |     |     |     |
| Asp Glu Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser Trp Pro |     |     |     |     |
| 654                                                         | 663 | 672 | 681 | 690 |
| CAC GAA GCA GCC AGC TCG TCC CAG CGT CGG CAG CCG CCC CCG GGC |     |     |     |     |
| His Glu Ala Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Gly Ser |     |     |     |     |
| 699                                                         | 708 | 717 | 726 | 735 |
| GCC GCG CAC CCG CTC AAC CGC AAG AGC CTT CTG GCC CCC GGA TCT |     |     |     |     |
| Pro Pro Gly Ala Ala His Pro Leu Asn Arg Lys Ser Leu Leu Ala |     |     |     |     |
| 744                                                         | 753 | 762 | 771 | 780 |
| GGC AGC GGC GGC GCG TCC CCA CTG ACC AGC GCG CAG GAC AGC GCC |     |     |     |     |
| Gly Ser Gly Gly Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala |     |     |     |     |
| 789                                                         | 798 | 807 | 816 | 825 |
| TTC CTC AAC GAC GCG GAC ATG GTC ATG AGC TTT GTG AAC CTG GTG |     |     |     |     |
| Phe Leu Asn Asp Ala Asp MET Val MET Ser Phe Val Asn Leu Val |     |     |     |     |
| 834                                                         | 843 | 852 | 861 | 870 |
| GAG TAC GAC AAG GAG TTC TCC CCT CGT CAG CGA CAC CAC AAA GAG |     |     |     |     |
| Glu Tyr Asp Lys Glu Phe Ser Pro Arg Gln Arg His His Lys Glu |     |     |     |     |
| 879                                                         | 888 | 897 | 906 | 915 |
| TTC AAG TTC AAC TTA TCC CAG ATT CCT GAG GGT GAG GTG GTG ACG |     |     |     |     |
| Phe Lys Phe Asn Leu Ser Gln Ile Pro Glu Gly Glu Val Val Thr |     |     |     |     |
| 924                                                         | 933 | 942 | 951 | 960 |
| GCT GCA GAA TTC CGC ATC TAC AAG GAC TGT GTT ATG GGG AGT TTT |     |     |     |     |
| Phe Arg Ile Tyr Lys Asp Cys Val MET Ala Ala Glu Gly Ser Phe |     |     |     |     |

SUBSTITUTE SHEET

12/32

## FIGURE 4C

969 978 987 996 1005  
 AAA AAC CAA ACT TTT CTT ATC AGC ATT TAT CAA GTC TTA CAG GAG  
 Lys Asn Gln Thr Phe Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu

1014 1023 1032 1041 1050  
 CAT CAG CAC AGA GAC TCT GAC CTG TTT TTG TTG GAC ACC CGT GTA  
 His Gln His Arg Asp Ser Asp Leu Phe Leu Leu Asp Thr Arg Val

1059 1068 1077 1086 1095  
 GTA TGG GCC TCA GAA GAA GGC TGG CTG GAA TTT GAC ATC ACG GCC  
 Val Trp Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp Ile Thr Ala

1104 1113 1122 1131 1140  
 ACT AGC AAT CTG TGG GTT GTG ACT CCA CAG CAT AAC ATG GGG CTT  
 Thr Ser Asn Leu Trp Val Val Thr Pro Gln His Asn MET Gly Leu

1149 1158 1167 1176 1185  
 CAG CTG AGC GTG GTG ACA AGG GAT GGA GTC CAC GTC CAC CCC CGA  
 Gln Leu Ser Val Val Thr Arg Asp Gly Val His Val His Pro Arg

1194 1203 1212 1221 1230  
 GCC GCA GGC CTG GTG GGC AGA GAC GGC CCT TAC GAT AAG CAG CCC  
 Ala Ala Gly Leu Val Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro

1239 1248 1257 1266 1275  
 TTC ATG GTG GCT TTC TTC AAA GTG AGT GAG GTC CAC GTG CGC ACC  
 Phe MET Val Ala Phe Phe Lys Val Ser Glu Val His Val Arg Thr

1284 1293 1302 1311 1320  
 ACC AGG TCA GCC TCC AGC CGG CGC CGA CAA CAG AGT CGT AAT CGC  
 Thr Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser Arg Asn Arg  
 (382)

1329 1338 1347 1356 1365  
 TCT ACC CAG TCC CAG GAC GTG GCG CGG GTC TCC AGT GCT TCA GAT  
Ser Thr Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala Ser Asp  
 (388)

1374 1383 1392 1401 1410  
 TAC AAC AGC AGT GAA TTG AAA ACA GCC TGC AGG AAG CAT GAG CTG  
 Tyr Asn Ser Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu Leu  
 (412)

1419 1428 1437 1446 1455  
 TAT GTG AGT TTC CAA GAC CTG GGA TGG CAG GAC TGG ATC ATT GCA  
Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala

SUBSTITUTE SHEET

## FIGURE 4D

|                 |                    |                 |             |            |
|-----------------|--------------------|-----------------|-------------|------------|
| 1464            | 1473               | 1482            | 1491        | 1500       |
| CCC AAG GGC TAT | GCT GCC AAT TAC    | TGT GAT GGA GAA | TGC TCC TTC |            |
| Pro Lys Gly Tyr | Ala Ala Asn Tyr    | Cys Asp Gly Glu | Cys Ser Phe |            |
| 1509            | 1518               | 1527            | 1536        | 1545       |
| CCA CTC AAC GCA | CAC ATG AAT GCA    | ACC AAC CAC GCG | ATT GTG CAG |            |
| Pro Leu Asn Ala | His MET Asn Ala    | Thr Asn His Ala | Ile Val Gln |            |
| 1554            | 1563               | 1572            | 1581        | 1590       |
| ACC TTG GTT CAC | CTT ATG AAC CCC    | GAG TAT GTC CCC | AAA CCG TGC |            |
| Thr Leu Val His | Leu MET Asn Pro    | Glu Tyr Val Pro | Lys Pro Cys |            |
| 1599            | 1608               | 1617            | 1626        | 1635       |
| TGT GCG CCA ACT | AAG CTA AAT GCC    | ATC TCG GTT CTT | TAC TTT GAT |            |
| Cys Ala Pro Thr | Lys Leu Asn Ala    | Ile Ser Val Leu | Tyr Phe Asp |            |
| 1644            | 1653               | 1662            | 1671        | 1680       |
| GAC AAC TCC AAT | GTC ATT CTG AAA    | AAA TAC AGG AAT | ATG GTT GTA |            |
| Asp Asn Ser Asn | Val Ile Leu Lys    | Lys Tyr Arg Asn | MET Val Val |            |
| 1689            | 1698               | 1708            | 1718        | 1728       |
| AGA GCT TGT GGA | TGC CAC TAACTCGAAA | CCAGATGCTG      | GGGACACACA  |            |
| Arg Ala Cys Gly | Cys His            |                 |             |            |
|                 | (513)              |                 |             |            |
| 1738            | 1748               | 1758            | 1768        | 1778       |
| TTCTGCCTTG      | GATTCCTAGA         | TTACATCTGC      | CTTAAAAAAA  | CACGGAAGCA |
| 1788            | 1798               | 1808            | 1818        | 1828       |
| CAGTTGGAGG      | TGGGACGATG         | AGACTTTGAA      | ACTATCTCAT  | GCCAGTGCCT |
| 1838            | 1848               | 1858            | 1868        | 1878       |
| TATTACCCAG      | GAAGATTTTA         | AAGGACCTCA      | TTAATAATTT  | GCTCACTTGG |
| 1888            | 1898               | 1908            | 1918        | 1928       |
| TAAATGACGT      | GAGTAGTTGT         | TGGTCTGTAG      | CAAGCTGAGT  | TTGGATGTCT |
| 1938            | 1948               | 1958            | 1968        | 1978       |
| GTAGCATAAG      | GTCTGGTAAC         | TGCAGAAACA      | TAACCGTGAA  | GCTCTTCCTA |
| 1988            | 1998               | 2008            | 2018        | 2028       |
| CCCTCCTCCC      | CCAAAAACCC         | ACCAAAATTA      | GTTTTAGCTG  | TAGATCAAGC |
| 2038            | 2048               | 2058            | 2068        | 2078       |
| TATTTGGGGT      | GTTTGTTAGT         | AAATAGGGAA      | AATAATCTCA  | AAGGAGTTAA |
| 2088            | 2098               | 2108            | 2118        | 2128       |
| ATGTATTCTT      | GGCTAAAGGA         | TCAGCTGGTT      | CAGTACTGTC  | TATCAAAGGT |

SUBSTITUTE SHEET

14/32

## FIGURE 4E

|            |            |            |            |            |
|------------|------------|------------|------------|------------|
| 2138       | 2148       | 2158       | 2168       | 2178       |
| AGATTTTACA | GAGAACAGAA | ATCGGGGAAG | TGGGGGGAAC | GCCTCTGTTC |
| 2188       | 2198       | 2208       | 2218       | 2228       |
| AGTTCATTCC | CAGAAGTCCA | CAGGACGCAC | AGCCCAGGCC | ACAGCCAGGG |
| 2238       | 2248       | 2258       | 2268       | 2278       |
| CTCCACGGGG | CGCCCTTGTC | TCAGTCATTG | CTGTTGTATG | TTCGTGCTGG |
| 2288       | 2298       | 2308       | 2318       | 2328       |
| AGTTTTGTG  | GTGTGAAAAT | ACACTTATTT | CAGCCAAAAC | ATACCATTTC |
| 2338       | 2348       | 2358       | 2368       | 2378       |
| TACACCTCAA | TCCTCCATTT | GCTGTACTCT | TTGCTAGTAC | CAAAAGTAGA |
| 2388       | 2398       | 2408       | 2418       | 2428       |
| CTGATTACAC | TGAGGTGAGG | CTACAAGGGG | TGTGTAACCG | TGTAACACGT |
| 2438       | 2448       | 2458       | 2468       | 2478       |
| GAAGGCAGTG | CTCACCTCTT | CTTTACCAGA | ACGGTTCTTT | GACCAGCACA |
| 2488       | 2498       | 2508       | 2518       | 2528       |
| TTAACTTCTG | GACTGCCGGC | TCTAGTACCT | TTTCAGTAAA | GTGGTTCTCT |
| 2538       | 2548       | 2558       | 2568       | 2578       |
| GCCTTTTAC  | TATACAGCAT | ACCACGCCAC | AGGGTTAGAA | CCAACGAAGA |
| 2588       | 2598       | 2608       | 2618       | 2628       |
| AAATAAAATG | AGGGTGCCCA | GCTTATAAGA | ATGGTGTTAG | GGGGATGAGC |
| 2638       | 2648       | 2658       | 2668       | 2678       |
| ATGCTGTTTA | TGAACGGAAG | TCATGATTTC | CCTGTAGAAA | GTGAGGCTCA |
| 2688       | 2698       | 2708       | 2718       | 2728       |
| GATTAAATTT | TAGAATATTT | TCTAAATGTC | TTTTTCACAA | TCATGTGACT |
| 2738       | 2748       | 2758       | 2768       | 2778       |
| GGGAAGGCAA | TTTCATACTA | AACTGATTAA | ATAATACATT | TATAATCTAC |
| 2788       | 2798       | 2808       | 2818       | 2828       |
| AACTGTTTGC | ACTTACAGCT | TTTTTTGTAA | ATATAAACTA | TAATTTATTG |
| 2838       | 2848       | 2858       | 2868       | 2878       |
| TCTATTTTAT | ATCTGTTTTG | CTGTGGCGTT | GGGGGGGGGG | CCGGGCTTTT |
| 2888       | 2898       | 2908       | 2918       |            |
| GGGGGGGGGG | GTTTGTGTTG | GGGGTGTCGT | GGTGTGGGCG | GGCGG      |

SUBSTITUTE SHEET

## FIGURE 5A

10 20 30 40 50  
CTGGTATATT TGTGCCTGCT GGAGGTGGAA TTAACAGTAA GAAGGAGAAA

60 70 80 90 100  
GGGATTGAAT GGA CTTACAG GAAGGATTTC AAGTAAATTC AGGGAAACAC

110 120 130 140 150  
ATTTACTTGA ATAGTACAAC CTAGAGTATT ATTTTACACT AAGACGACAC

160 170 180 190 200  
AAAAGATGTT AAAGTTATCA CCAAGCTGCC GGACAGATAT ATATTCCAAC

210 220 230 240 250  
ACCAAGGTGC AGATCAGCAT AGATCTGTGA TTCAGAAATC AGGATTTGTT

260 270 280 290 300  
TTGGAAAGAG CTCAAGGGTT GAGAAGA ACT CAAAAGCAAG TGAAGATTAC

310 320 330 340 350  
TTTGGGA ACT ACAGTTTATC AGAAGATCAA CTTTGTCTAA TTCAAATACC

360 370 380 390 400  
AAAGGCCTGA TTATCATAAA TTCATATAGG AATGCATAGG TCATCTGATC

410 420 430 440 450  
AAATAATATT AGCCGTCTTC TGCTACATCA ATGCAGCAAA AACTCTTAAC

460 470 480 490 500  
AACTGTGGAT AATTGGAAAT CTGAGTTTCA GCTTTCTTAG AAATAACTAC

510 520 530 540 550  
TCTTGACATA TTCCAAAATA TTTAAAATAG GACAGGAAAA TCGGTGAGGA

560 570 580 590 600  
TGTTGTGCTC AGAAATGTCA CTGTCATGAA AAATAGGTAA ATTTGTTTTT

610 620 630 640 650  
TCAGCTACTG GGAAACTGTA CCTCCTAGAA CCTTAGGTTT TTTTTTTTTT

660 670 680 690 700  
AAGAGGACAA GAAGGACTAA AAATATCAAC TTTTGCTTTT GGACAAAA

SUBSTITUTE SHEET

16/32

## FIGURE 5B

|                                                             |      |      |      |      |
|-------------------------------------------------------------|------|------|------|------|
| 701                                                         | 710  | 719  | 728  | 737  |
| ATG CAT CTG ACT GTA TTT TTA CTT AAG GGT ATT GTG GGT TTC CTC |      |      |      |      |
| MET His Leu Thr Val Phe Leu Leu Lys Gly Ile Val Gly Phe Leu |      |      |      |      |
| (1)                                                         |      |      |      |      |
| 746                                                         | 755  | 764  | 773  | 782  |
| TGG AGC TGC TGG GTT CTA GTG GGT TAT GCA AAA GGA GGT TTG GGA |      |      |      |      |
| Trp Ser Cys Trp Val Leu Val Gly Tyr Ala Lys Gly Gly Leu Gly |      |      |      |      |
| 791                                                         | 800  | 809  | 818  | 827  |
| GAC AAT CAT GTT CAC TCC AGT TTT ATT TAT AGA AGA CTA CGG AAC |      |      |      |      |
| Asp Asn His Val His Ser Ser Phe Ile Tyr Arg Arg Leu Arg Asn |      |      |      |      |
| 836                                                         | 845  | 854  | 863  | 872  |
| CAC GAA AGA CGG GAA ATA CAA AGG GAA ATT CTC TCT ATC TTG GGT |      |      |      |      |
| His Glu Arg Arg Glu Ile Gln Arg Glu Ile Leu Ser Ile Leu Gly |      |      |      |      |
| 881                                                         | 890  | 899  | 908  | 917  |
| TTG CCT CAC AGA CCC AGA CCA TTT TCA CCT GGA AAA ATG ACC AAT |      |      |      |      |
| Leu Pro His Arg Pro Arg Pro Phe Ser Pro Gly Lys Gln Ala Ser |      |      |      |      |
| 926                                                         | 935  | 944  | 953  | 962  |
| CAA GCG TCC TCT GCA CCT CTC TTT ATG CTG GAT CTC TAC AAT GCC |      |      |      |      |
| Ser Ala Pro Leu Phe MET Leu Asp Leu Tyr Asn Ala MET Thr Asn |      |      |      |      |
| 971                                                         | 980  | 989  | 998  | 1007 |
| GAA GAA AAT CCT GAA GAG TCG GAG TAC TCA GTA AGG GCA TCC TTG |      |      |      |      |
| Glu Glu Asn Pro Glu Glu Ser Glu Tyr Ser Val Arg Ala Ser Leu |      |      |      |      |
| 1016                                                        | 1025 | 1034 | 1043 | 1052 |
| GCA GAA GAG ACC AGA GGG GCA AGA AAG GGA TAC CCA GCC TCT CCC |      |      |      |      |
| Ala Glu Glu Thr Arg Gly Ala Arg Lys Gly Tyr Pro Ala Ser Pro |      |      |      |      |
| 1061                                                        | 1070 | 1079 | 1088 | 1097 |
| AAT GGG TAT CCT CGT CGC ATA CAG TTA TCT CGG ACG ACT CCT CTG |      |      |      |      |
| Asn Gly Tyr Pro Arg Arg Ile Gln Leu Ser Arg Thr Thr Pro Leu |      |      |      |      |
| 1106                                                        | 1115 | 1124 | 1133 | 1142 |
| ACC ACC CAG AGT CCT CCT CTA GCC AGC CTC CAT GAT ACC AAC TTT |      |      |      |      |
| Thr Thr Gln Ser Pro Pro Leu Ala Ser Leu His Asp Thr Asn Phe |      |      |      |      |
| 1151                                                        | 1160 | 1169 | 1178 | 1187 |
| CTG AAT GAT GCT GAC ATG GTC ATG AGC TTT GTC AAC TTA GTT GAA |      |      |      |      |
| Leu Asn Asp Ala Asp MET Val MET Ser Phe Val Asn Leu Val Glu |      |      |      |      |
| 1196                                                        | 1205 | 1214 | 1223 | 1232 |
| AGA GAC AAG GAT TTT TCT CAC CAG CGA AGG CAT TAC AAA GAA TTT |      |      |      |      |
| Arg Asp Lys Asp Phe Ser His Gln Arg Arg His Tyr Lys Glu Phe |      |      |      |      |

SUBSTITUTE SHEET

## FIGURE 5C

|                                                                    |      |      |      |       |
|--------------------------------------------------------------------|------|------|------|-------|
| 1241                                                               | 1250 | 1259 | 1268 | 1277  |
| CGA TTT GAT CTT ACC CAA ATT CCT CAT GGA GAG GCA GTG ACA GCA        |      |      |      |       |
| Arg Phe Asp Leu Thr Gln Ile Pro His Gly Glu Ala Val Thr Ala        |      |      |      |       |
| 1286                                                               | 1295 | 1304 | 1313 | 1322  |
| GCT GAA TTC CGG ATA TAC AAG GAC CGG AGC AAC AAC CGA TTT GAA        |      |      |      |       |
| Ala Glu Phe Arg Ile Tyr Lys Asp Arg Ser Asn Asn Arg Phe Glu        |      |      |      |       |
| 1331                                                               | 1340 | 1349 | 1358 | 1367  |
| AAT GAA ACA ATT AAG ATT AGC ATA TAT CAA ATC ATC AAG GAA TAC        |      |      |      |       |
| Asn Glu Thr Ile Lys Ile Ser Ile Tyr Gln Ile Ile Lys Glu Tyr        |      |      |      |       |
| 1376                                                               | 1385 | 1394 | 1403 | 1412  |
| ACA AAT AGG GAT GCA GAT CTG TTC TTG TTA GAC ACA AGA AAG GCC        |      |      |      |       |
| Thr Asn Arg Asp Ala Asp Leu Phe Leu Leu Asp Thr Arg Lys Ala        |      |      |      |       |
| 1421                                                               | 1430 | 1439 | 1448 | 1457  |
| CAA GCT TTA GAT GTG GGT TGG CTT GTC TTT GAT ATC ACT GTG ACC        |      |      |      |       |
| Gln Ala Leu Asp Val Gly Trp Leu Val Phe Asp Ile Thr Val Thr        |      |      |      |       |
| 1466                                                               | 1475 | 1484 | 1493 | 1502  |
| AGC AAT CAT TGG GTG ATT AAT CCC CAG AAT AAT TTG GGC TTA CAG        |      |      |      |       |
| Ser Asn His Trp Val Ile Asn Pro Gln Asn Asn Leu Gly Leu Gln        |      |      |      |       |
| 1511                                                               | 1520 | 1529 | 1538 | 1547  |
| CTC TGT GCA GAA ACA GGG GAT GGA CGC AGT ATC AAC GTA AAA TCT        |      |      |      |       |
| Leu Cys Ala Glu Thr Gly Asp Gly Arg Ser Ile Asn Val Lys Ser        |      |      |      |       |
| 1556                                                               | 1565 | 1574 | 1583 | 1592  |
| GCT GGT CTT GTG GGA AGA CAG GGA CCT CAG TCA AAA CAA CCA TTC        |      |      |      |       |
| Ala Gly Leu Val Gly Arg Gln Gly Pro Gln Ser Lys Gln Pro Phe        |      |      |      |       |
| 1601                                                               | 1610 | 1619 | 1628 | 1637  |
| ATG GTG GCC TTC TTC AAG GCG AGT GAG GTA CTT CTT CGA TCC GTG        |      |      |      |       |
| MET Val Ala Phe Phe Lys Ala Ser Glu Val Leu Leu Arg Ser Val        |      |      |      |       |
| 1646                                                               | 1655 | 1664 | 1673 | 1682  |
| AGA GCA GCC AAC AAA CGA AAA AAT CAA AAC CGC AAT AAA TCC AGC        |      |      |      |       |
| Arg Ala Ala Asn Lys Arg Lys Asn Gln Asn Arg Asn Lys <u>Ser Ser</u> |      |      |      |       |
|                                                                    |      |      |      | (329) |
| 1691                                                               | 1700 | 1709 | 1718 | 1727  |
| TCT CAT CAG GAC TCC TCC AGA ATG TCC AGT GTT GGA GAT TAT AAC        |      |      |      |       |
| <u>Ser His Gln Asp Ser Ser Arg MET Ser Ser Val Gly Asp Tyr Asn</u> |      |      |      |       |
|                                                                    |      |      |      | (337) |

**FIGURE 5D**

# SUBSTITUTE SHEET

Figure 6

(1)  
GAATTCC GAG CCC CAT TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT  
Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala  
(10)

GGG GAG GCG GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC  
Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His  
(20) (30)

CTG CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC  
Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser  
(40) (50)

AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT GGA GAC  
Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp  
(60) (70)

GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC TGG TTG CTG AAG  
Glu Gly Typ Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu Lys  
(80)

CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG ACT GAG GAT GGG CAC AGC  
Arg His Lys Asp Leu Gly Lue Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser  
(90) (100)

GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT CAA CGG GCC CCA CGC TCC CAA CAG  
Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln  
(110) (120)

CCT TTC GTG GTC ACT TTC TTC AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG  
Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg  
(130) (140)

GCA GTG AGG CCA CTG AGG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG  
Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln  
(150) (160)

GCC AAC CGA CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG  
Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln  
(170)

GTC TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTT GGC TGG CTG GAC  
Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp  
(180) (190)

TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAG GGG GAG TGC TCC  
Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser  
(200) (210)

TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG  
Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu  
(220) (230)

SUBSTITUTE SHEET

## Figure 6 (Con't)

GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG TGC TGT GCA CCC ACC AAG  
Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys  
(240) (250)

CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC AGC AGC AAC AAC GTC ATC CTG CGC  
Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg  
(260)

AAG CAC CGC AAC ATG GTG GTC AAG GCC TGC GGC TGC CAC TGAGTCAGCCCCGCCAGC  
Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
(270) (280)

CCTACTGCAGCCACCCTTCTCATCTGGATCGGGCCCTGCAGAGGCAGAAAACCCTTAAATGCTGTCACAG  
CTCAAGCAGGAGTGTCAGGGGCCCTCACTCTCGGTGCCTACTTCCTGTCAGGCTTCTGGGAATTC

**SUBSTITUTE SHEET**

FIGURE 7

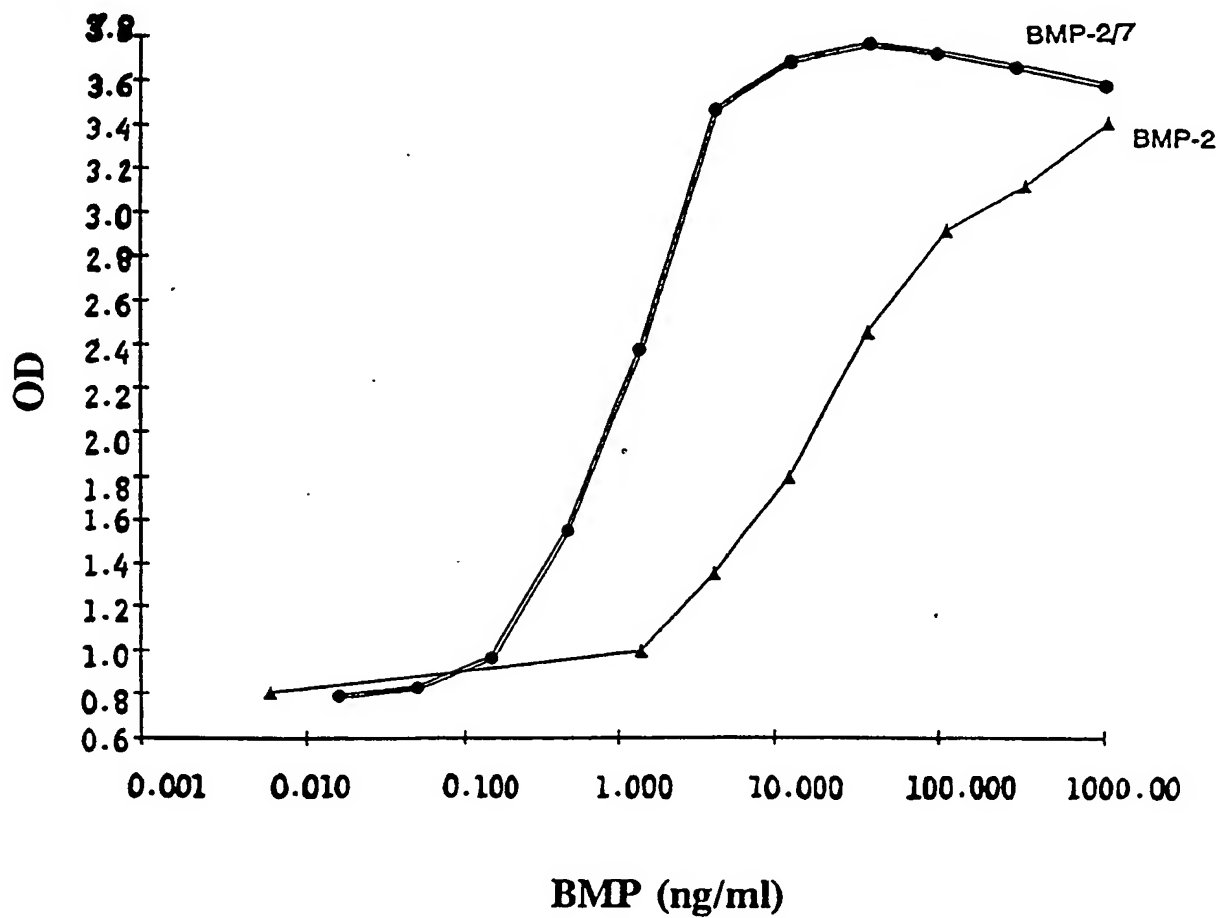
GACGAAAGGG CCTCCTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT 60  
 CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT 120  
 TCTAAATACA TTCAAATATG TATCGGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT 180  
 AATATTGAAA AAGGAAAGAGT ATGAGTATTC AACATTTGCG TGTGGCCCTT ATTCCCTTTT 240  
 TTGCGGCAAT TTGCTTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAGATG 300  
 CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAAGT GATCTCAAC AGCGGTAAAG 360  
 TCCTTGAGAG TTTTCGCCCC GAAGAAGGTT TTCCAATGAT GAGCACTTTT AAGTTCTGEC 420  
 TATGTGGCGC GGTATTATCC CGTATTGAGC CCGGGCAAGA GCAACTCGGT CGCGGCATAC 480  
 ACTATTGTCA GAATGACTTG GTTGAGTACT CACCACTGAC AGAAAAGCAT CTTACGGATG 520  
 GCATGACAGT AAGAGAATTA TGCAGTCTG CGATAACCAT GAGTGATAAC ACTGCGGCCA 600  
 ACTTACTTCT GACAAAGATC GGAAGAGCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG 660  
 GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GATGAAGCC ATACCAAAAG 720  
 ACGAGCGTGA CACCACGATG CCGTAGCAA TGGCAACAAC GTTCCGCAAA CTATTAACTG 780  
 GCGAAGTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG 840  
 TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGCTG GTTTATTGCT GATAAATCTG 900  
 GAGCCCGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAAGCCCT 960  
 CCCGTATGCT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC 1020  
 AGATCGCTGA GATAGCTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT 1080  
 CATATATACT TTAGATTGAT TTAAGACTTC ATTTTAAATT TAAAGGATC TAGGTGAGA 1140  
 TCCTTTTTGA TAATCTCATG ACCAAAATCC GTTAACGTGA GTTTTCGTTT CACTGAGCGT 1200  
 CAGACCCCGT AGAAAAGATC AAGGATCTT GTTGAGATCC TTTTTTCTG GCGTAATCT 1260  
 GCTGCTTGA AACAAAAA CCACCGCTAC CAGCGGTGCT TTGTTGCCG GATCAAGAGC 1320  
 TACCAACTCT TTTTCGAAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGCTC 1380  
 TTCTAGTGA GCCGTAGTTA GCGCACCACT TCAAGAACTC TGTAGCAGCG COTACATACC 1440  
 TCGGTCTGCT AATGCTGTTA CGAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG 1500  
 GGTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTGCGGCTGA ACGGGCGGTT 1560  
 CGTGACACA GCCCAGCTTG GAGCGAAGCA COTAGACCGA ACTGAGATAC CTACAGCGTG 1620  
 AGCATTGAGA AAGCGCCACC CTTCCGAAAG GAGAGAAAGC GACAGGTAT CCGGTAAAGC 1680  
 GCAGGGTCGG AACAGGAGAG CGCACGAGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT 1740  
 ATAGTCTGT CGGGTTTGG CACCTGTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG 1800  
 GGGGGCGGAG CCTATGAAA AACGCCAGCA AGCGGGCGTT TTTACGGTTC CTGGCCTTTT 1860  
 GCTGGCCTTT TGCTCACATG TTCTTCCCTG CGTTATCCCC TGATTCTGTG GATAACCOTA 1920

SUBSTITUTE SHEET

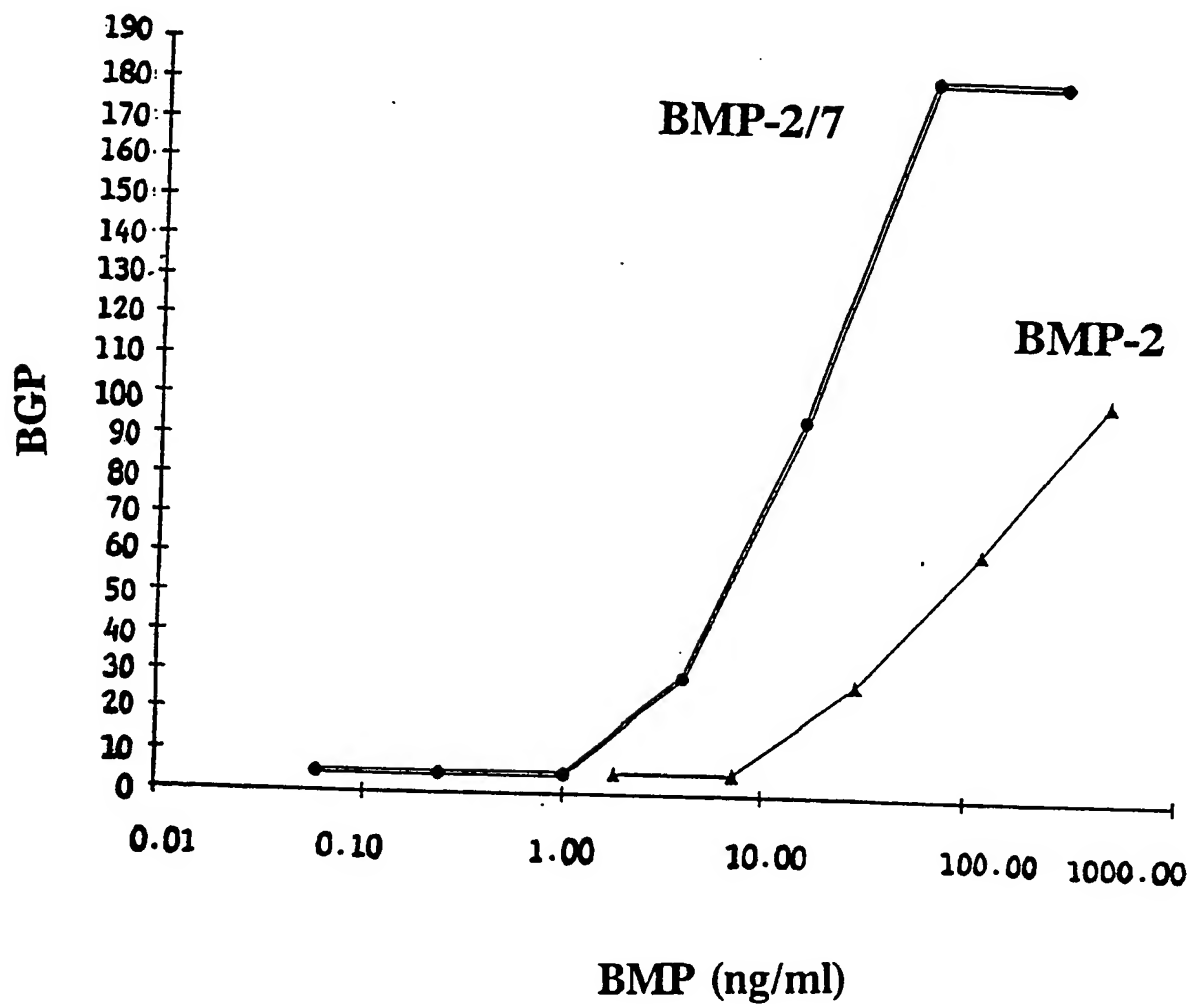
FIGURE 7 (cont'd)

TTACCGCCTT TGAOTGAGCT GATACCGCTC GCGGCAGCCG AACGACCGAG CGCAGCGAGT 1980  
 CAGTGAGCGA GGAAGCGGAA GAGCGGCCAA TACGCAAAAC GCCTCTCCCC GCGCGTTGGC 2040  
 CGATTCAATTA ATGCAGAATT GATCTCTCAC CTACCAAAACA ATGCCCCCCT GCAAAAAATA 2100  
 AATTCATATA AAAAACATAC AGATAACCAT CTGCGGTGAT AAATTATCTC TCGCGGTGTT 2160  
 GACATAAATA CCACTGGCGG TGATACTGAG CACATCAGCA GGACGCACTG ACCACCATGA 2220  
 AGGTGACGCT CTAAAAATT AAGCCCTGAA GAAGGCGAGC ATTCAAAGCA GAAAGCTTTG 2280  
 GCGTGTCTGA TACGAAACGA AGCATTGGCC GTAAAGTGGG TTCCGATTA GCTGCCAATG 2340  
 TCGCAATCGC GGGGCGTTT CGTTCAGGAC TACAACTGCC ACACACCACC AAAGCTAACT 2400  
 GACAGGAGAA TCCAGATGA TGCACAAACA CGCGCCGCGC AACGTCCGCG AGAGAAACAG 2460  
 GCTCAATGGA AAGCAGCAAA TCCCCTGTTG GTTGGGGTAA GCGCAAAAC AGTTCCGAAA 2520  
 GATTTTTTTA ACTATAAACG CTGATGGAAG CGTTTATGCG GAAAGGTTAA AGCCCTTCCC 2580  
 CAGTAACAAA AAAACAACAG CXTAAATAAC CCGCTCTTA CACATTCCAG CCCTGAAAAA 2640  
 GGGCATCAA TTAACCACA CCTATGGTGT ATGCATTTAT TTGCATACAT TCAATCAATT 2700  
 GTTATCTAAG GAAATACTTA CATATGCAAG CTAAACATAA ACAACGTAAA CGTCTGAAT 2760  
 CTAGCTGTAA GAGACAGCCT TTGTACGTGG ACTTCAGTGA CGTGGGTTGG AATGACTGGA 2820  
 TTGTGGCTCC CCGCGGTAT CACGCTTTT ACTGCCAGG AGAATGCCCT TTTCCTCTGG 2880  
 CTGATCATCT GAACTCCACT AATCATGCCA TTGTTCAAGC GTTGGTCAAC TCTGTTAACT 2940  
 CTAAGATTCC TAAGGCATGC TGTGTCCGA CAGAACTCAG TGCTATCTCG ATGCTGTACC 3000  
 TTGACGAGAA TGAAGGTT GTATTAAAG ACTATCAGGA CATGTTGTG GAGGGTTGTG 3060  
 GGTOTCGCTA GTACAGCAA ATTAAATACA TAAATATATA TATATATATA TATTTTAGAA 3120  
 AAAAGAAAAA AATCTAGAGT CGACCTGCAG TAATCCTACA GGGTAGTACA AATAAAAAAG 3180  
 GCACGTCAGA TGACGTGCTT TTTTCTTGT GAGCAGTAAG CTTGGCACTG GCGCTCGTTT 3240  
 TACAACGTGG TGACTGGGAA AACCTGGCG TTACCCAAGT TAATCGCCTT GCAGCACATC 3300  
 CCCCCTTCCG CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT 3360  
 TGCGCAGCCT GAATGCGGAA TGGCGCTCA TCGGTATTT TCTCCTTACG CATCTGTGCG 3420  
 GTATTTTACA CCOCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT 3480  
 AAGCCAGCCC CGACACCCGC CAACACCCGC TGACGCGCCC TGACGGGCTT GTCTGCTCCG 3540  
 GGCATCCGCT TACAGACAAG CTGTGACCGT CTCCGGAGC TGCAATGTCT AGAGGTTTTT 3600  
 ACCGTCATCA CCGAAACCGG CGA 3623

SUBSTITUTE SHEET

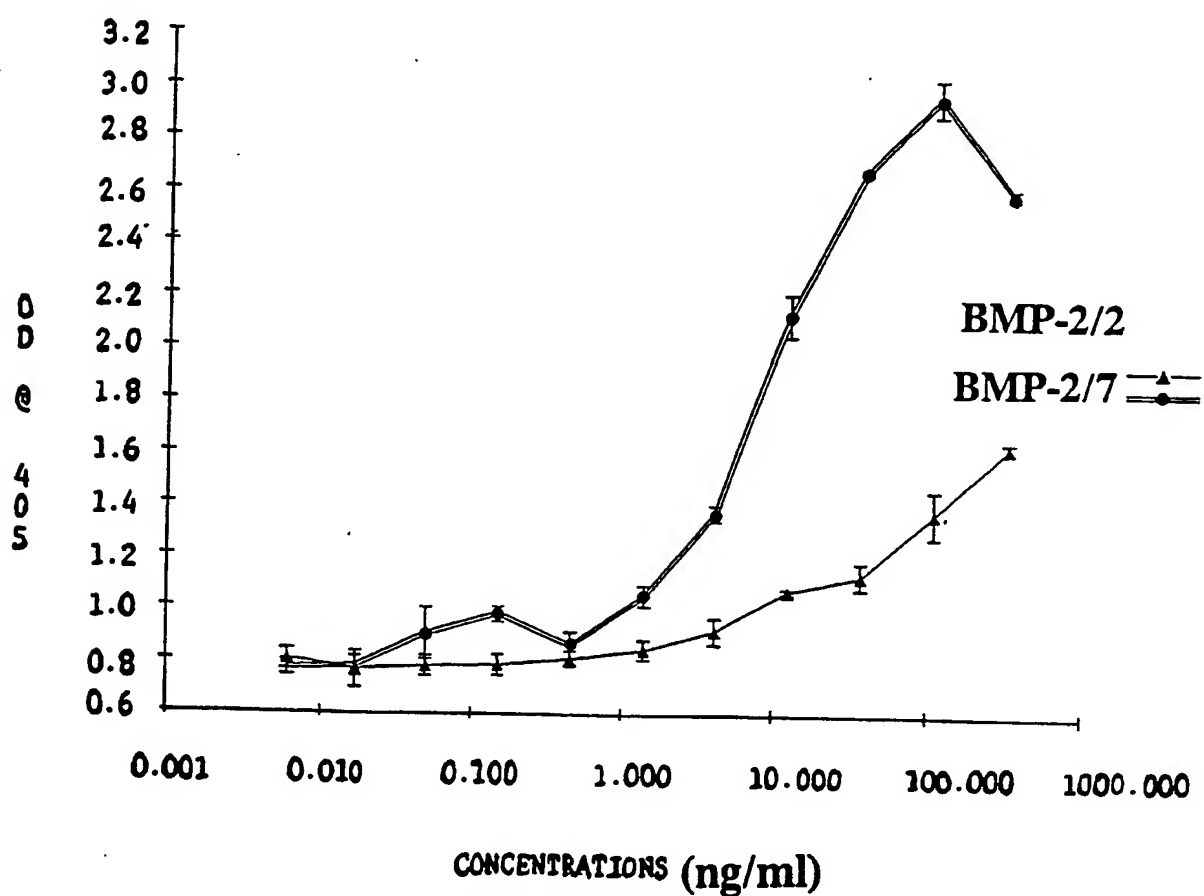
**FIGURE 8****W-20 ALKALINE PHOSPHATASE: BMP-2 VS. BMP-2/7**

24/32

**FIGURE 9****EFFECTS OF BMP-2 AND BMP2/7 ON BGP SYNTHESIS BY W-20 CELLS**

SUBSTITUTE SHEET

25/32

**FIGURE 10****COMPARAISON OF *E. Coli* BMP-2 AND BMP-2/7:  
W-20-17 ALKALINE PHOSPHATASE**

SUBSTITUTE SHEET

## FIGURE 11A

10 20 30 40 50 60 70  
 AGATCTTGAA AACACCCGGG OCACACAAGC OGOGAOCCTAC AGCTCTTTCT CAGOGTTGGA GTGGAGAOGG  
 80 90 100 110 120 130 140  
 OGCCCGCAGC GCGCTGCGCG GGTGAGGTCC GCGCAGCTGC TGGGGAAGAG CCGACCTGTC AGGCTGCGCT  
 150 160 170 180 190 200 210  
 GGGTCAGCGC AGCAAGTGGG GCTGCGCGCT ATCTGCGTGC ACGCGGCGCG GTCCCGGGCT CCGTGGCGCC  
 220 230 240 250 260 270 280  
 TCGCCCGCAGC TGGTTTGGAG TTCAACCTTC GGCTCCGCGG CCGGCTCTTT GCGCTTCCG AGTGTCGCGC  
 290 300 310 320 (1) 335  
 AGCGAAGCGG GGAGCGGAAG GCGCGCGCGG GTACCTAGCC ATG GCT GGG GCG AGC AGG CTG CTC  
 MET Ala Gly Ala Ser Arg Leu Leu  
 350 365 380 395  
 TTT CTG TGG CTG GGC TGC TTC TGC GTG AGC CTG GCG CAG GGA GAG AGA CCG AAG CCA  
 Phe Leu Trp Leu Gly Cys Phe Cys Val Ser Leu Ala Gln Gly Glu Arg Pro Lys Pro  
 410 425 440 455  
 CCT TTC CCG GAG CTC GCG AAA GCT GTG CCA GGT GAC GCG ACG GCA GGT GGT GGC CCG  
 Pro Phe Pro Glu Leu Arg Lys Ala Val Pro Gly Asp Arg Thr Ala Gly Gly Gly Pro  
 470 485 500 515  
 GAC TCC GAG CTG CAG CCG CAA GAC AAG GTC TCT GAA CAC ATG CTG CCG CTC TAT GAC  
 Asp Ser Glu Leu Gln Pro Gln Asp Lys Val Ser Glu His MET Leu Arg Leu Tyr Asp  
 530 545 560  
 AGG TAC AGC ACG GTC CAG GCG GCG CCG ACA CCG GGC TCC CTG GAG GGA GGC TCG CAG  
 Arg Tyr Ser Thr Val Gln Ala Ala Arg Thr Pro Gly Ser Leu Glu Gly Gly Ser Gln  
 575 590 605 620  
 CCC TGG CCG CCT CCG CTC CTG CCG GAA GGC AAC ACG GTT CCG AGC TTT CCG GCG GCA  
 Pro Trp Arg Pro Arg Leu Leu Arg Glu Gly Asn Thr Val Arg Ser Phe Arg Ala Ala  
 635 650 665 680  
 GCA GCA GAA ACT CTT GAA AGA AAA GGA CTG TAT ATC TTC AAT CTG ACA TCG CTA ACC  
 Ala Ala Glu Thr Leu Glu Arg Lys Gly Leu Tyr Ile Phe Asn Leu Thr Ser Leu Thr  
 695 710 725 740  
 AAG TCT GAA AAC ATT TTG TCT GCC ACA CTG TAT TTC TGT ATT GGA GAG CTA GGA AAC  
 Lys Ser Glu Asn Ile Leu Ser Ala Thr Leu Tyr Phe Cys Ile Gly Glu Leu Gly Asn

SUBSTITUTE SHEET

27/32

## FIGURE 11C

1430                      1445      (377)                      1460                      1475  
 TGC GGC AGG AGA TAC CTC AAG GTA GAC TTT GCA GAT ATT GGC TGG AGT GAA TGG ATT  
 Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser Glu Trp Ile

1490                      1505                      1520                      1535  
 ATC TOC CCC AAG TOC TTT GAT GOC TAT TAT TGC TCT GGA GCA TGC CAG TTC CCC ATG  
 Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly Ala Cys Gln Phe Pro MET

1550                      1565                      1580                      1595  
 CCA AAG TCT TTG AAG CCA TCA AAT CAT GCT ACC ATC CAG AGT ATA GTG AGA GCT GTG  
 Pro Lys Ser Leu Lys Pro Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val

1610                      1625                      1640                      1655  
 GGG GTC GTT OCT GGG ATT OCT GAG OCT TGC TGT GTA CCA GAA AAG ATG TOC TCA CTC  
 Gly Val Val Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Glu Lys MET Ser Ser Leu

1670                      1685                      1700  
 AGT ATT TTA TTC TTT GAT GAA AAT AAG AAT GTA GTG CTT AAA GTA TAC OCT AAC ATG  
 Ser Ile Leu Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn MET

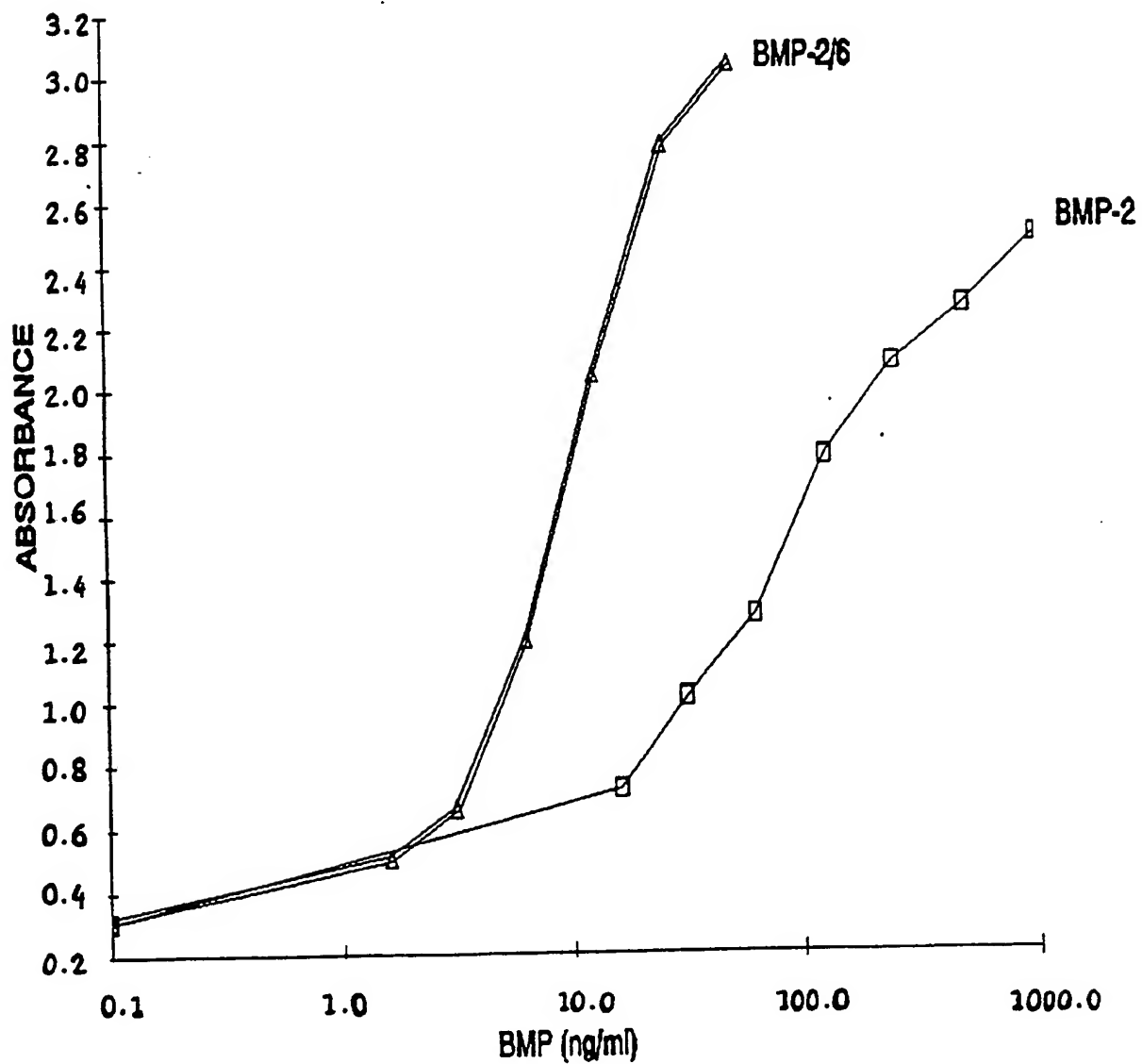
1715                      1730      (472)                      1746                      1756                      1766                      1776  
 ACA GTA GAG TCT TGC GCT TGC AGA TAACCTGGCA AAGAACTCAT TTGAATGCTT AATTCAATCT  
Thr Val Glu Ser Cys Ala Cys Arg

1786  
 CTAGAGTGA CGGAATTC

SUBSTITUTE SHEET

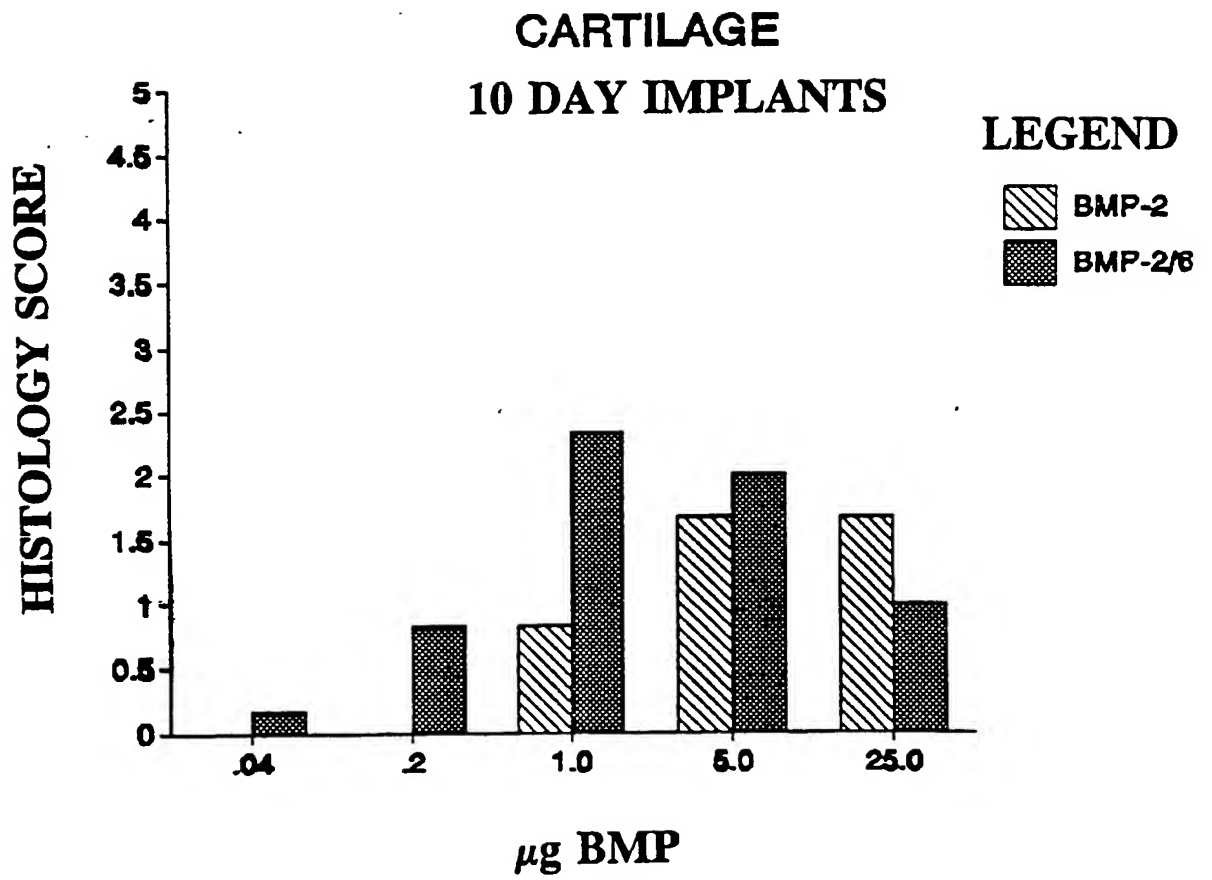
Figure 12

W-20 ALKALINE PHOSPHATASE: CHO BMP-2/6 vs. CHO BMP-2



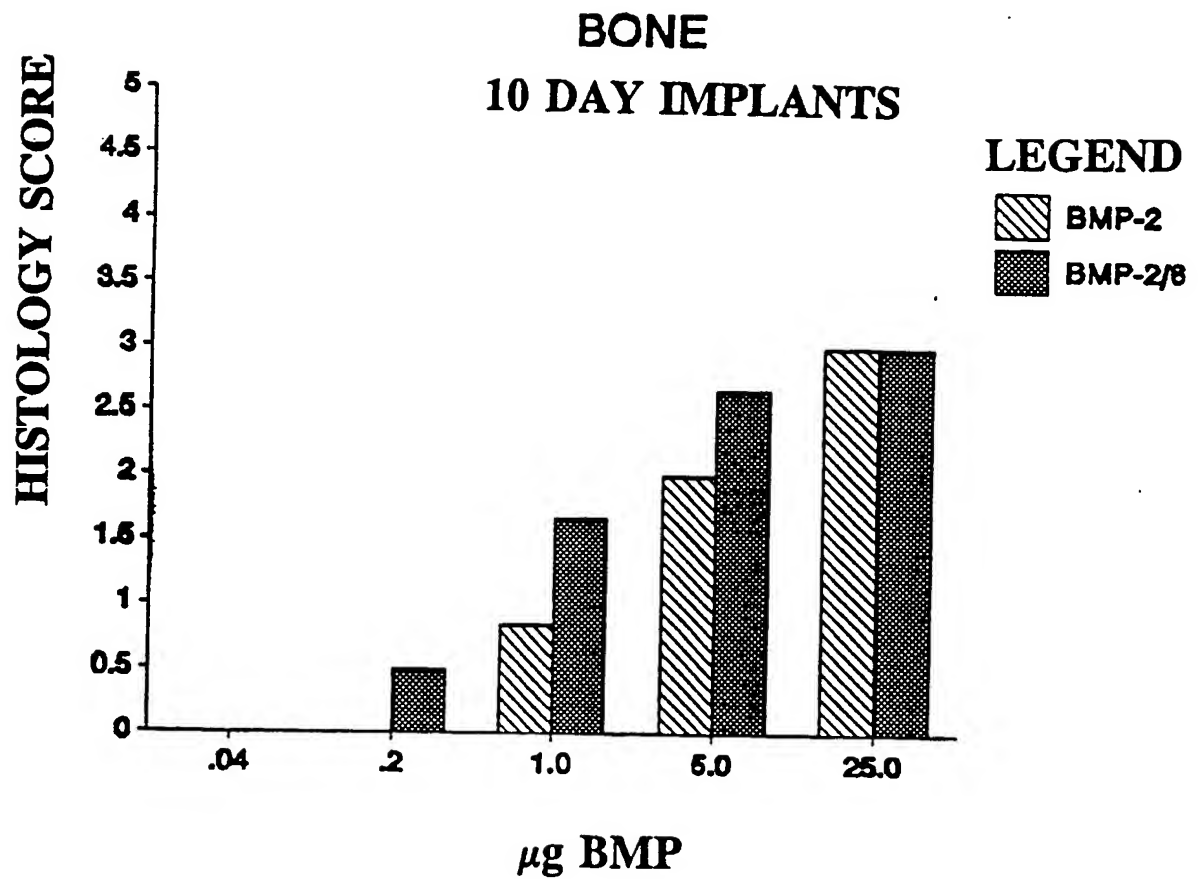
SUBSTITUTE SHEET

29/32

**FIGURE 13A**

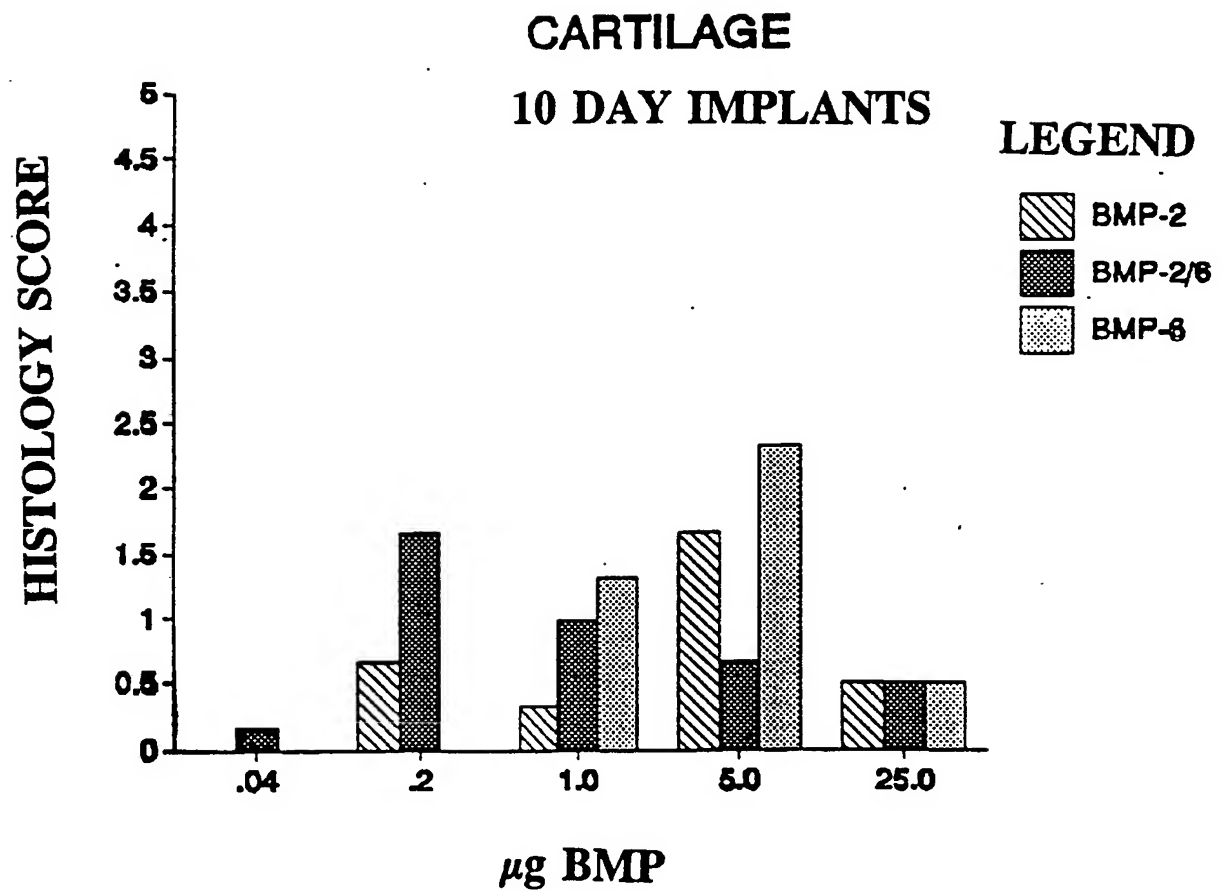
SUBSTITUTE SHEET

30/32

**FIGURE 13B**

SUBSTITUTE SHEET

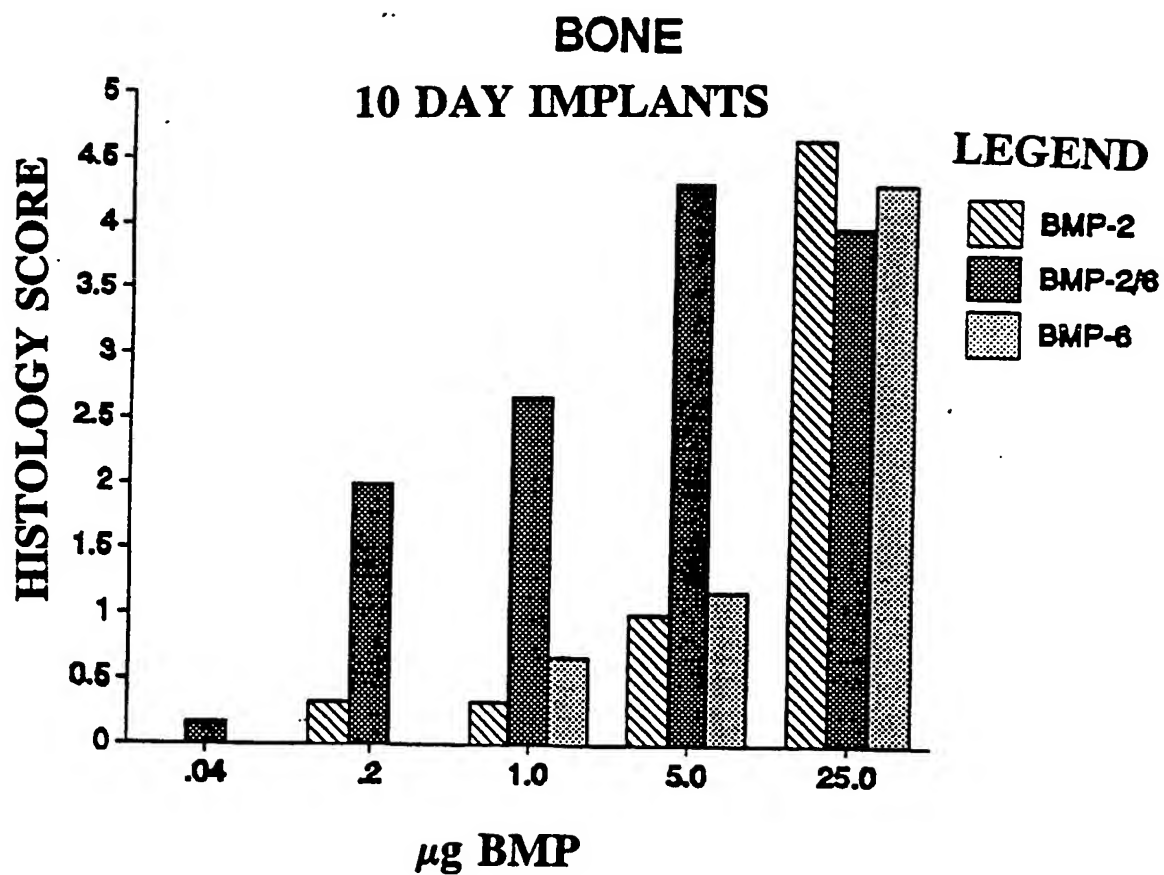
31/32

**FIGURE 14A**

SUBSTITUTE SHEET

32/32

FIGURE 14B



SUBSTITUTE SHEET

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/09430

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                    |                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|
| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                    |                                                                   |
| According to International Patent Classification (IPC) or to both National Classification and IPC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                    |                                                                   |
| Int.Cl. 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | C12N15/12;<br>C07K15/06                                                                                                                                            | C12P21/02; A61K37/02; C12N5/12                                    |
| <b>II. FIELDS SEARCHED</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                    |                                                                   |
| Minimum Documentation Searched <sup>7</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                    |                                                                   |
| Classification System                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Classification Symbols                                                                                                                                             |                                                                   |
| Int.Cl. 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | C07K ; C12N ; A61K ; C12P                                                                                                                                          |                                                                   |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                    |                                                                   |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                    |                                                                   |
| Category <sup>10</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>                                                     | Relevant to Claim No. <sup>13</sup>                               |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | WO,A,9 003 733 (INTERNATIONAL GENETIC ENGINEERING, INC.)<br>19 April 1990<br>see page 16, line 7 - page 17, line 28<br>see page 18, line 22 - line 34              | 1,4,<br>7-14,16,<br>23-26                                         |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | see page 51, line 32 - page 52, line 10;<br>figure 12<br>see page 62 - page 63; claim 35<br>---                                                                    | 13-17,<br>33,35                                                   |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | WO,A,9 011 366 (GENETICS INSTITUTE, INC.)<br>4 October 1990<br>cited in the application<br>see page 22, line 20 - line 27<br>see page 43, line 17 - line 30<br>--- | 13-16,33                                                          |
| -/--                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                    |                                                                   |
| <p><sup>10</sup> Special categories of cited documents :<sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> |                                                                                                                                                                    |                                                                   |
| <b>IV. CERTIFICATION</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                    |                                                                   |
| Date of the Actual Completion of the International Search<br><br>04 FEBRUARY 1993                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                    | Date of Mailing of this International Search Report<br>26. 02. 93 |
| International Searching Authority<br><br>EUROPEAN PATENT OFFICE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                    | Signature of Authorized Officer<br><br>ANDRES S.M.                |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |                                                                                                                                                                                                                                                                                                                                                                                                                         |                           |
|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| Category *                                                                 | Citation of Document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                      | Relevant to Claim No.     |
| X                                                                          | WO,A,8 910 409 (GENETICS INSTITUTE, INC.)<br>2 November 1989<br>cited in the application<br>see page 7, line 13 - line 15<br>see page 8, line 20 - line 29<br>---                                                                                                                                                                                                                                                       | 1,4,<br>7-12,23,<br>25-26 |
| X                                                                          | PROCEEDINGS OF THE NATIONAL ACADEMY OF<br>SCIENCES OF USA<br>vol. 87, March 1990, WASHINGTON US<br>pages 2220 - 2224<br>WANG, E.A. ET AL. 'Recombinant human bone<br>morphogenetic protein induces bone<br>formation'<br>cited in the application<br>see figure 1C<br>---                                                                                                                                               | 34,36                     |
| Y                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                         | 35                        |
| X                                                                          | JOURNAL OF BIOLOGICAL CHEMISTRY<br>vol. 265, no. 22, 5 August 1990,<br>BALTIMORE, MD US<br>pages 13198 - 13205<br>SAMPATH, T.K. ET AL. 'Bovine osteogenic<br>protein is composed of dimers of OP-1 and<br>BMP-2A, two members of the transforming<br>growth factor-beta superfamily'<br>see the whole document<br>---                                                                                                   | 34,36                     |
| X                                                                          | PROCEEDINGS OF THE NATIONAL ACADEMY OF<br>SCIENCES OF USA<br>vol. 87, no. 24, December 1990, WASHINGTON<br>US<br>pages 9843 - 9847<br>CELESTE, A.J. ET AL. 'Identification of<br>transforming growth factor-beta family<br>members present in bone-inductive protein<br>purified from bovine bone'<br>see page 9846, left column, line 13 -<br>right column, line 7<br>see page 9847, left column, paragraph 2-3<br>--- | 34,36                     |
| A                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                         | 13,16                     |
| A                                                                          | WO,A,8 909 787 (CREATIVE BIOMOLECULES,<br>INC.)<br>19 October 1989<br>see page 6, line 22 - line 24<br>see page 56, paragraphs E5 & E6<br>---                                                                                                                                                                                                                                                                           |                           |
| P,Y                                                                        | WO,A,9 118 098 (GENETICS INSTITUTE, INC.)<br>28 November 1991<br>cited in the application<br>see page 12, line 31 - page 13, line 7<br>---                                                                                                                                                                                                                                                                              | 17                        |
|                                                                            | ---<br>-/--                                                                                                                                                                                                                                                                                                                                                                                                             |                           |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |                                                                                                                                                                                                                                                                                                                                                                                 |                       |
|----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category <sup>a</sup>                                                      | Citation of Document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                              | Relevant to Claim No. |
| P,X                                                                        | <p>JOURNAL OF CELLULAR BIOCHEMISTRY<br/>Supplement 16F, 1992, page 76,<br/>abstract W026; WOZNEY, J.M. ET AL.:<br/>'Regulation of chondrogenesis and<br/>osteogenesis by the BMP proteins'<br/>see abstract<br/>&amp; Keystone Symposium on growth and<br/>differentiation factors in vertebrate<br/>development; Keystone, Colorado, USA<br/>April 3-16, 1992</p> <p>-----</p> | 1                     |

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9209430  
SA 66918

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 04/02/93

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| WO-A-9003733                              | 19-04-90            | US-A- 5106626              | 21-04-92            |
|                                           |                     | AU-B- 615810               | 10-10-91            |
|                                           |                     | AU-A- 4488689              | 01-05-90            |
|                                           |                     | CA-A- 2000498              | 11-04-90            |
|                                           |                     | EP-A- 0394418              | 31-10-90            |
|                                           |                     | JP-T- 4505151              | 10-09-92            |
| WO-A-9011366                              | 04-10-90            | US-A- 5106748              | 21-04-92            |
|                                           |                     | US-A- 5141905              | 25-08-92            |
|                                           |                     | AU-A- 5357790              | 22-10-90            |
|                                           |                     | CA-A- 2030518              | 29-09-90            |
|                                           |                     | EP-A- 0429570              | 05-06-91            |
|                                           |                     | JP-T- 3505098              | 07-11-91            |
| WO-A-8910409                              | 02-11-89            | AU-A- 3448789              | 24-11-89            |
|                                           |                     | EP-A- 0408649              | 23-01-91            |
|                                           |                     | JP-T- 3503649              | 15-08-91            |
|                                           |                     | US-A- 5106748              | 21-04-92            |
| WO-A-8909787                              | 19-10-89            | US-A- 4968590              | 06-11-90            |
|                                           |                     | US-A- 5011691              | 30-04-91            |
|                                           |                     | AU-B- 628050               | 10-09-92            |
|                                           |                     | AU-A- 3444989              | 03-11-89            |
|                                           |                     | AU-B- 618357               | 19-12-91            |
|                                           |                     | AU-A- 3530589              | 03-11-89            |
|                                           |                     | EP-A- 0372031              | 13-06-90            |
|                                           |                     | EP-A- 0362367              | 11-04-90            |
|                                           |                     | JP-T- 3500655              | 14-02-91            |
|                                           |                     | JP-T- 3502579              | 13-06-91            |
|                                           |                     | WO-A- 8909788              | 19-10-89            |
|                                           |                     | US-A- 5108753              | 28-04-92            |
|                                           |                     | AU-B- 627850               | 03-09-92            |
|                                           |                     | AU-A- 5174790              | 26-09-90            |
|                                           |                     | EP-A- 0411105              | 06-02-91            |
|                                           |                     | JP-T- 3504736              | 17-10-91            |
|                                           |                     | WO-A- 9010018              | 07-09-90            |
|                                           |                     | US-A- 4975526              | 04-12-90            |
|                                           |                     | US-A- 5171574              | 15-12-92            |
|                                           |                     | US-A- 5162114              | 10-11-92            |

ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.

US 9209430  
SA 66918

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

04/02/93

Page 2

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| WO-A-9118098                              | 28-11-91            | None                       |                     |

EPO FORM P0079

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82